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(57) Abstract

The present invention provides novel α-O-linked glycoconjugates such as α-O-linked glycopeptides, as well as convergent methods for synthesis thereof. The general preparative approach is exemplified by the synthesis of the mucin motif commonly found on epithelial tumor cell surfaces. The present invention further provides compositions and methods of treating cancer using the α-O-linked glycoconjugates.

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TRIMERIC ANTIGENIC O-LINKED GLYCOPEPTIDE CONJUGATES, METHODS OF PREPARATION AND USES THEREOF

This application is based on U.S. Provisional Application Serial No. 60/079,312, filed March 25, 1998, the contents of which are hereby incorporated by reference into this application. This invention was made with government support under grants CA-28824, HL-25848 and AI-16943 from the National Institutes of Health. Accordingly, the U.S. Government has certain rights in the invention.

Field of the Invention

The present invention is in the field of α-O-linked glycopeptides. In particular, the present invention relates to methods for the preparation of α-O-linked glycoconjugates with clustered glycodomains which are useful as anticancer therapeutics. The present invention also provides novel compositions comprising such α-O-linked glycoconjugates and methods for the treatment of cancer using these glycoconjugtes.

Throughout this application, various publications are referred to, each of which is hereby incorporated by reference in its entirety into this application to more fully describe the state of the art to which the invention pertains.

Background of the Invention

The role of carbohydrates as signaling molecules in the context of biological processes has recently gained prominence. M.L. Phillips, *et al., Science*, **1990**, *250*, 1130; M.J. Polley, *et al., Proc. Natl. Acad. Sci. USA*, **1991** *88*, 6224: T. Taki, *et al., J. Biol. Chem.*, **1996**, *261*, 3075; Y. Hirabayashi, A. Hyogo, T. Nakao, K. Tsuchiya, Y. Suzuki, M. Matsumoto, K. Kon, S. Ando, ibid., **1990**, *265*, 8144; O. Hindsgaul, T. Norberg, J. Le Pendu, R.U. Lemieux, *Carbohydr. Res.* **1982**, *109*, 109; U. Spohr, R.U. Lemieux, *ibid.*, **1988**, *174*, 211). The elucidation of the scope of carbohydrate involvement in mediating cellular interaction is an important area of inquiry in contemporary biomedical research.

glycoconjugates (cf. glycoproteins and glycolipids) rather than as free entities. Given the complexities often associated with isolating the conjugates in homogeneous form and the difficulties in retrieving intact carbohydrates from these naturally occurring conjugates, the applicability of synthetic approaches is apparent. (For recent reviews of glycosylation see: Paulsen, H.; *Angew. Chemie Int. Ed. Engl.* **1982**, *21*, 155; Schmidt, R.R., *Angew. Chemie Int. Ed. Engl.* **1986**, *25*, 212; Schmidt, R.R., *Comprehensive Organic Synthesis*, Vol. 6, Chapter 1(2), Pergamon Press, Oxford, **1991**; Schmidt, R.R., *Carbohydrates, Synthetic Methods and Applications in Medicinal Chemistry*, Part I, Chapter 4, VCH Publishers, Weinheim, New York, **1992**. For the use of glycals as glycosyl donors in glycoside synthesis, see Lemieux, R.U., *Can. J. Chem.*, **1964**, *42*, 1417; Lemieux, R.U., Fraiser-Reid, B., Can. *J. Chem.* **1965**, *43*, 1460; Lemieux, R.U.; Morgan, A.R., Can. *J. Chem.* **1965**, *43*, 2190; Thiem, J., *et al., Synthesis* **1978**, 696; Thiem, J. Ossowski, P., *Carbohydr. Chem.*, **1984**, *3*, 287; Thiem, J., *et al., Liebigs Ann. Chem.*, **1986**, 1044; Thiem, J. in *Trends in Synthetic Carbohydrate Chemistry*, Horton, D., *et al.*, eds., ACS Symposium Series No. 386, American Chemical Society, Washington, D.C., **1989**, Chapter 8.)

The carbohydrate domains of the blood group substances contained in both glycoproteins and glycolipids are distributed in erythrocytes, epithelial cells and various secretions. The early focus on these systems centered on their central role in determining blood group specificities. R.R. Race; R. Sanger, Blood Groups in Man, 6th ed., Blackwell, Oxford, **1975**. However, it is recognized that such determinants are broadly implicated in cell adhesion and binding phenomena. (For example, see M.L. Phillips, *et al., Science* **1990**, *250*, 1130.) Moreover, ensembles related to the blood group substances in conjugated form are encountered as markers for the onset of various tumors. K.O. Lloyd, *Am. J. Clinical Path.*, **1987**, *87*, 129; K.O. Lloyd, *Cancer Biol.*, **1991**, *2*, 421. Carbohydrate-based tumor antigenic factors have applications at the diagnostic level, as resources in drug delivery or ideally in immunotherapy. Toyokuni, T., *et al., J. Am. Chem Soc.* **1994**, *116*, 395; Dranoff, G., et *al., Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3539; Tao, M-H.; Levy, R., *Nature* **1993**, *362*, 755; Boon, T., *Int. J. Cancer* **1993**, *54*, 177; Livingston, P.O., *Curr. Opin. Immunol.* **1992**, *4*, 624; Hakomori, S., *Annu. Rev. Immunol.* **1984**, *2*, 103; K. Shigeta, *et al., J. Biol. Chem.* **1987**, *262*, 1358.

The present invention provides new strategies and protocols for glycopeptide synthesis. The object is to simplify such preparations so that relatively complex domains can be assembled with high stereospecifity. Major advances in glycoconjugate synthesis require the attainment of a high degree of convergence and relief from the burdens associated with the manipulation of blocking groups. Another requirement is that of delivering the carbohydrate determinant with appropriate provision for conjugation to carrier proteins or lipids. Bernstein, M.A.; Hall, L.D., *Carbohydr. Res.*

1980, *78*, Cl; Lemieux, R.U., *Chem. Soc. Rev.* **1978**, *7*, 423; R.U. Lemieux, et al., *J. Am. Chem. Soc.* **1975**, *97*, 4076. This is a critical condition if the synthetically derived carbohydrates are to be incorporated into carriers suitable for clinical application.

Antigens which are selective (or ideally specific) for cancer cells could prove useful in fostering active immunity. Hakomori, S., *Cancer Res.*, **1985**, *45*, 2405-2414; Feizi, T., *Cancer Surveys* **1985**, *4*, 245-269. Novel carbohydrate patterns are often presented by transformed cells as either cell surface glycoproteins or as membrane-anchored glycolipids. In principle, well chosen synthetic glycoconjugates which stimulate antibody production could confer active immunity against cancers which present equivalent structure types on their cell surfaces. Dennis, J., *Oxford Glycostems Glyconews*, Second Ed., **1992**; Lloyd, K.O., in *Specific Immunotherapy of Cancer with Vaccines*, **1993**, New York Academy of Sciences, pp.50-58. Chances for successful therapy improve with increasing restriction of the antigen to the target cell. For example, one such specific antigen is the glycosphingolipid isolated by Hakomori and collaborators from the breast cancer cell line MCF-7 and immunocharacterized by monoclonal antibody MBrl. Bremer, E.G., *et al., J. Biol. Chem.* **1984**, *259*, 14773-14777; Menard, S., *et al., Cancer Res.* **1983**, *43*, 1295-1300.

The surge of interest in glycoproteins (M.J. McPherson, *et al.*, eds., *PCR A Practical Approach*, **1994**, Oxford University Press, Oxford, G.M. Blackburn; M.J. Gait, Eds., *Nucleic Acids in Chemistry and Biology*, **1990**, Oxford University Press, Oxford; A.M. Bray; A.G. Jhingran; R.M. Valero; N.J. Maeji, *J. Org. Chem.* **1944**, *59*, 2197; G. Jung; A.G. Beck-Sickinger, *Angew Chem. Int. Ed. Engl.* **1992**, *31*, 367; M.A. Gallop; R.W. Barrett; W.J. Dower; S.P.A. Fodor; E.M. Gordon, *J. Med. Chem.* **1994**, *37*, 1233; H.P. Nestler; P.A. Bartlett; W.C. Still, *J. Org. Chem.* **1994**, *59*, 4723; M. Meldal, *Curr. Opin. Struct. Biol.* **1994**, *4*, 673) arises from heightened awareness of their importance in diverse biochemical processes including cell growth regulation, binding of pathogens to cells (O.P. Bahl, in *Glycoconjugates: Composition, structure, and function*, H. J. Allen, E.C. Kisailus, Eds., **1992**, Marcel Dekker, Inc., New York, p. 1), intercellular communication and metastasis (A. Kobata, *Acc. Chem. Res.* **1993**, *26*, 319). Glycoproteins serve as cell differentiation markers and assist in protein folding and transport, possibly by providing protection against proteolysis. G. Opdenakker, *et al., FASEB J.* **1993**, *7*, 1330. Improved isolation techniques and structural elucidation methods (A. De; K.-H. Khoo, *Curr. Opin. Struct. Biol.* **1993**, *3*, 687) have revealed high levels of microheterogeneity in naturally-produced glycoproteins. R.A. Dwek, *et al., Annu. Rev. Biochem.* **1993**, *62*, 65. Single eukaryotic cell lines often produce many glycoforms of any given protein sequence. For instance, erythropoietin (EPO), a clinically useful red blood cell stimulant against anemia, is glycosylated by more than 13 known types of oligosaccharide chains when expressed in

Chinese hamster ovary cells (CHO) (Y.C. Lee; R.T. Lee, Eds., *Neoglycoconjugates: Preparation and Applications*, **1994**, Academic Press, London). The efficacy of erythropoietin is heavily dependent on the type and extent of glycosylation (E. Watson, *et al.*, *Glycobiology*, **1994**, *4*, 227).

Elucidation of the biological relevance of particular glycoprotein oligosaccharide chains requires access to pure entities, heretofore obtained only by isolation. Glycoprotein heterogeneity renders this process particularly labor-intensive. However, particular cell lines can be selected to produce more homogeneous glycoproteins for structure-activity studies. U.S. Patent No. 5,272,070. However, the problem of isolation from natural sources remains difficult.

Receptors normally recognize only a small fraction of a given macromolecular glycoconjugate. Consequently, synthesis of smaller but well-defined putative glycopeptide ligands could emerge as competitive with isolation as a source of critical structural information (Y.C. Lee; R.T. Lee, Eds., *supra*).

Glycoconjugates prepared by total synthesis are known to induce mobilization of humoral responses in the murine immune system. Ragupathi, G., et *al.*, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 125; Toyokuni, T.; Singhal, A.K., *Chem. Soc. Rev.* **1995**, *24*, 231; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1381. Glycopeptides, in contrast to most glycolipids and carbohydrates themselves, are known to bind to major histocompatability complex (MHC) molecules and stimulate T cells in favorable cases. Deck, B., *et al.*, *J. Immunology* **1995**, 1074; Haurum, J.S., *et al.*, *J. Exp. Med.* **1994**, *180*, 739; Sieling, P.A., et *al.*, *Science* **1995**, *269*, 227 (showing T cell recogniztion of CD1-restricted microbial glycolipid). Properly stimulated T cells express receptors that specifically recognize the carbohydrate portion of a glycopeptide. The present invention demonstrates a means of augmenting the immunogenicity of carbohydrates by use of a peptide attachment.

Preparation of chemically homogeneous glycoconjugates, including glycopeptides and glycoproteins, constitutes a challenge of high importance. Bill, R.M.; Flitsch, S.L.; *Chem. & Biol.* **1996**, *3*, 145. Extension of established cloning approaches to attain these goals are being actively pursued. Various expression systems (including bacteria, yeast and cell lines) provide approaches toward this end, but, as noted above, produce heterogeneous glycoproteins. Jenkins, N., *et al.*, *Nature Biotech.* **1996**, *14*, 975. Chemical synthesis thus represents a preferred avenue to such bi-domainal constructs in homogeneous form. Moreover, synthesis allows for the assembly of constructs in which selected glycoforms are incorporated at any desired position of the peptide chain.

Prior to the subject invention, methods of glycopeptide synthesis pioneered by Kunz and others allowed synthetic access to homogenous target systems

both in solution and solid phase (M. Meldal, *Curr. Opin. Struct. Biol*, **1994**, *4*, 710; M. Meldal, in *Neoglycoconjugates: Preparation and Applications, supra*; S.J. Danishefsky; J.Y. Roberge, in *Glycopeptides and Related Compounds: Chemical Synthesis, Analysis and Applications*, **1995**, D.G. Large, C.D. Warren, Eds., Marcel Dekker, New York; S.T. Cohen-Anisfeld and P.T. Lansbury, Jr., *J. Am. Chem. Soc.*, **1993**, *115*, 10531; S.T. Anisfeld; P.T. Lansbury Jr., *J. Org. Chem*, **1990**, *55*, 5560; D. Vetter, *et al., Angew. Chem. Int. Ed. Engl*, **1995**, *34*, 60-63). Cohen-Anisfeld and Lansbury disclosed a convergent solution-based coupling of selected already available saccharides with peptides. S.T. Cohen-Anisfeld; P.T. Lansbury, Jr., *J. Am. Chem. Soc., supra*.

Thus, few effective methods for the preparation of α-O-linked glycoconjugates were known prior to the present invention. Nakahara, Y., *et al.*, In *Synthetic Oligosaccharides*, ACS Symp. Ser. 560, **1994**, pp. 249-266; Garg, H.G., *et al., Adv. Carb. Chem. Biochem.* **1994**, *50*, 277. Nearly all approaches incorporated the amino acid (serine or threonine) at the monosaccharide stage. This construction would be followed by elaboration of the peptidyl and carbohydrate domains in a piecemeal fashion. Qui, D.; Koganty, R.R.; *Tetrahedron Lett.* **1997**, *38*, 45. Eloffson, M., *et al., Tetrahedron* **1997**, *53*, 369. Meinjohanns, E., *et al., J. Chem. Soc., Perkin Trans. 1*, **1996**, 985. Wang, Z-G., *et al., Carbohydr. Res.* **1996**, *295*, 25. Szabo, L., *et al., Carbohydr. Res.* **1995**, *274*, 11. The scope of the synthetic problem is well known in the art, but little progress has been achieved. The present invention provides an alternate, simpler and more convergent approach (Figure 2).

Toyokuni *et al., J.Amer.Chem.Soc.*, **1994**, *116*, 395, have prepared synthetic vaccines comprising dimeric Tn antigen-lipopeptide conjugates having efficacy in eliciting an immune response against Tn-expressing glycoproteins. However, prior to investigations of the present inventors, it was not appreciated that the surface of prostate cancer cells presents glycoproteins comprising Tn clusters linked via threonine rather than serine residues. Accordingly, the present invention provides a vaccine having unexpectedly enhanced anticancer efficacy.

Summary of the Invention

Accordingly, one object of the present invention is to provide novel α-O-linked glycoconjugates including glycopeptides and related compounds which are useful as anticancer therapeutics.

Another object of the present invention is to provide synthetic methods for preparing such glycoconjugates. An additional object of the invention is to provide compositions useful in the treatment of subjects suffering from cancer comprising any of the glycoconjugates available through the preparative methods of the invention, optionally

the glycoconjugates available through the preparative methods of the invention, optionally in combination with pharmaceutical carriers.

The present invention is also intended to provide a fully synthetic carbohydrate vaccine capable of fostering active immunity in humans.

A further object of the invention is to provide methods of treating subjects suffering from of cancer using any of the glycoconjugates available through the preparative methods of the invention, optionally in combination with pharmaceutical carriers.

Brief Description of the Drawings

Figure 1 shows a schematic structure for α-O-linked glycoconjugates as present in mucins.

Figure 2A-B provides a general synthetic strategy to mucin glycoconjugates.

Figure 3 provides a synthetic route to prepare key intermediate β-phenylthioglycoside **11**. Reaction conditions: (a) (1) DMDO, CH_2Cl_2; (2) 6-O-TIPS-galactal, $ZnCl_2$, -78°C to 0°C; (3) Ac_2O, Et_3N, DMAP, 75%; (b) TBAF/AcOH/THF; 80%; (c) 5 (1.3 eq), TMSOTf (0.1 eq), THF:Toluene 1:1, -60°C to -45°C, 84%, α:β 4:1; (d) NaN_3, CAN, CH_3CN, -15°C, 60%; (e) LiBr, CH_3CN, 75%; (f) (1) 1 PhSH, iPr_2NEt, CH_3CN, 82% (2) CCl_3CN, K_2CO_3, CH_2Cl_2, 80%; (g) (1) PhSH, iPr_2NEt; (2) $ClP(OEt)_2$, iPr_2NEt, THF, (labile compd, -72% for two steps); (h) (1) LiBr, CH_3CN, 75%; (2) LiSPh, THF, 0°C, 70%).

Figure 4A-B presents a synthetic route to glycoconjugate mucin **1**.
Reaction conditions: (a) CH_3COSH, 78%; (b) H_2 / 10% Pd-C, MeOH, H_20, quant.; (c) H_2N-Ala-Val-OBn, IIDQ, CH_2Cl_2, 85%; (d) KF, DMF, 18-crown-6, 95%; (e) **15**, IIDQ, 87%; (f) KF, DMF, 18-crown-6,93%; (g) **14**, IIDQ, 90%; (h) (1) KF, DMF, 18-crown-6; (2) Ac_2O, CH_2Cl_2,; (i) H_2 / 10% Pd-C, MeOH, H_20, 92% (three steps); (j) NaOH, H_20, 80%.,

Figure 5A-B shows a synthetic route to prepare glycoconjugates by a fragment coupling. Reagents: (a) IIDQ, CH_2Cl_2, rt, 80%; (b) H_2/Pd-C, MeOH, H_2O, 95%; (c) CF_3COOH, CH_2Cl_2; (d) NaOH, H_2O, MeOH.

Figure 6 shows the synthesis of α-O-linked glycopeptide conjugates of the Le^y epitope via an iodosulfonamidation/4 + 2 route.

Figure 7A-B provides the synthesis of α-O-linked glycopeptide conjugates of the Le^y epitope via an azidonitration/4 + 2 route.

Figures 8A-E and **9A-C** present examples of glycopeptides derived by the method of the invention.

Figure 10A-B illustrates a synthetic pathway to prepare glycopeptides ST_N and T(TF).

Figure 11A-B shows a synthetic pathway to prepare glycopeptide (2,3)ST.

Figure 12A-B shows a synthetic pathway to prepare the glycopeptide glycophorine.

Figure 13A-B presents a synthetic pathway to prepare glycopeptides 3-Le^y and 6-Le^y.

Figure 14A-C provides a synthetic pathway to prepare T-antigen.

Figure 15A-C shows a synthetic pathway to prepare the alpha cluster of the T-antigen.

Figure 16 shows a synthetic pathway to prepare the beta cluster of the T-antigen. The sequence of reactions are as represented in Figure 15.

Figures 17A-C, 18A-C and **19A-B** presents a synthesis of α-O-linked glycopeptide conjugates of the Le^y epitope. R is defined in Figure 18.

Figure 20 shows **(A)** the conjugation of Tn-trimer glycopeptide to PamCys lipopeptide; **(B)** a general representation of a novel vaccine construct; and **(C)** a PamCys Tn Trimer.

Figure 21 illustrates **(A)** a method of synthesis of a PamCys-Tn-trimer **3**; and **(B-D)** a method of preparation of KLH and BSA conjugates **(12, 13)** via cross-linker conjugation.

Figure 22 shows **(A)** a mucin related F1α antigen and a retrosynthetic approach to its preparation; and **(B)** a method of preparing intermediates **5'** and **6'**. conditions: i) NaN_3, CAN, CH_3, CN, -20 °C, overnight, 40%, α (**4a'**): β (**4b'**) 1:1; ii) PhSH, $EtN(i\text{-}Pr)_2$, CH_3,CN, 0 °C, 1h, 99.8%, iii) K_2CO_3, CCl_3,CN, CH_2Cl_2, rt, 5h, 84%, **5a'**: **5b'**(1:5;iv) DAST, CH_2Cl_2, 0 °C, 1h, 93%, **6a'**: **6b'** 1:1.

Figure 23 shows a method of preparing intermediates **1'** and **2'**. Conditions: i) TBAF, HOAc, THF, rt, 3d, 100% yield for **9'**, 94% yield for **10'**; ii) **11'**, $BF_3{\cdot}Et_2O$, -30 °C, overnight; iii) AcSH, pyridine, rt, overnight, 72% yield based on 50% conversion of **11'**, 58% yield based on 48% conversion of **12'** (two steps); iv) 80% aq. HOAc, overnight,

rt-40 °C; v) Ac_2O, pyridine, rt., overnight; vi) 10% Pd/C, H_2, MeOH-H_2O, rt, 4h; vii) morpholine, DMF, rt, overnight; viii) NaOMe, MeOH-THF, rt, overnight, 64% yield for **1´**, 72% yield for **2´** (five steps).

Figure 24 shows a method of preparing intermediates in the synthesis of F1α antigen. Conditions: i) (sym-collidine)$_2ClO_4$, $PhSO_2NH_2$, 0 °C; LiHMDS < EtSH, -40 °C-rt, 88% yield in two steps; ii) MeOTf, DTBP, 0 °C, 86% yield for 20´ plus 8% yield of α isomer; 85% yield for **21´** plus 6% yield of α isomer; iii) Na, NH_3, 78° C; Ac_2O_2, Py, rt, for **22´**, 59% yield in two steps; iv) NaN_3, CAN, CH_3CN, -20 ° C; v) PhSH, EtN(i-Pr)$_2$; CCl_3CN, K_2CO_3; for **23´**, 17 % yield of 2:7, α/β in three steps; for **24´** 30% yield of 3;1, α/β in three steps; vi) LiBr, CH_3CN, for **25´**, 46% yield, α only; vii) Ac_2O, Py; Na-Hg, Na_2HPO_4, 94% yield in two steps, NaN_3, CAN, 26% yield, PhSH, EtN(i-Pr)$_2$; K_2CO_3, CCl_3CN, 53% yield in two steps (**27´**); viii) LiSPh, THF, 60% yield, β only (**26´**).

Figure 25A-B shows a synthesis of a glycoconjugate containing a Le^y hexasaccharide.

Figure 26 shows a preparation of an intermediate to make a glycopeptide containing a TF antigen. Conditions: (a) DMDO, CH_2Cl_2, 0°C; (b) **19**, $ZnCl_2$, THF, -78°C to rt, 97%; (c) i) 80% AcOH, 70°C; ii) Ac_2O, DMAP, TEA, CH_2Cl_2, 93%; (d) $CH_3C(O)SH$, 19 h, 87%; (e) Pd/C, H_2, 2 h, quant.; (f) HOAt, HATU, collidine, DMF, 84%.

Figure 27 shows a preparation of a glycopeptide containing a TF antigen. Conditions: (a) KF, DMF, 48 h, 72-82%; (b) **47**, HOAt, HATU, collidine, DMF, 75-84%; (c) Ac_2O, CH_2Cl_2; (d) TFA, CH_2Cl_2; (e) SAMA-OPfp, DIEA, CH_2Cl_2; (f) NaOMe, MeOH (degassed), rt, 60%.

Figure 28A-C shows the synthesis of the hexasaccharide-based Le^y-containing lipoglycopeptide construct **6A** via the cassette strategy.

Figure 29A-B shows (a) O-linked pentasaccharide Le^y-containing monomers P_a and P_β and (b) pentasaccharide-based Le^y-containing lipoglycopeptide constructs **7A-9A**.

Figure 30 shows the reactivity of synthetic Le^y-hexa- and penta-saccharide lipoglycopeptides with mouse anti-Le^y monoclonal antibody 3S193 determined by ELISA. ♦: Compound **6A**; ■ : Compound **7A**; ▲ : Compound **8A**; ▾ : compound **9A**; • : Le^y-ceramide (**10A**).

Figure 31A-F shows the reactivity of sera from mice immunized with Le^y-pentasaccharide lipoglycopeptides with Le^y-ceramide (A, B, C) and Le^y/Le^b-expressing ovarian cyst mucin (D, E, F) determined by ELISA. A and D: mice immunized with **7A** (a-linked trimeric Le^y); B and E: mice immunized with **8A** (b-linked trimeric Le^y); C and F: mice immunized with **9A** (a-linked Le^y-monomer). Five female mice (Balb/c) were immunized in each group with lipoglycopeptides (containing 10 μg carbohydrate) in Intralipid (15 μL; Clintec Nutrition Co.) by a subcutaneous injection every week for 4 weeks and then at 9 weeks. Sera were obtained 10 days after the final immunization.

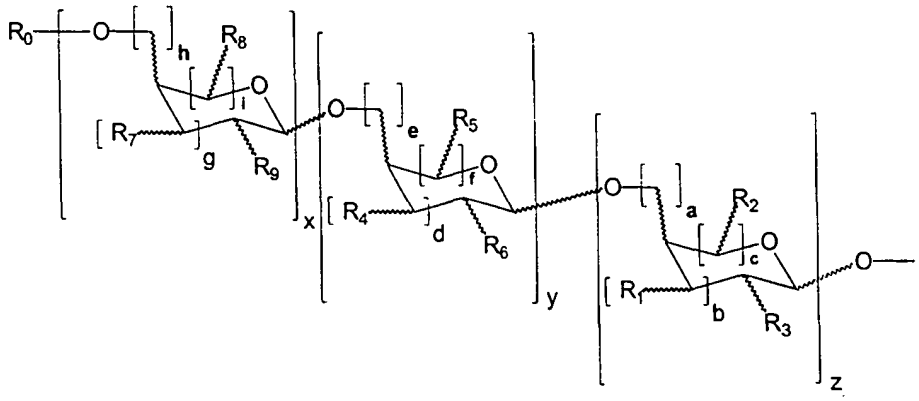
Detailed Description of the Invention

The subject invention provides novel α-O-linked glycoconjugates, useful in the prevention and treatment of cancer.

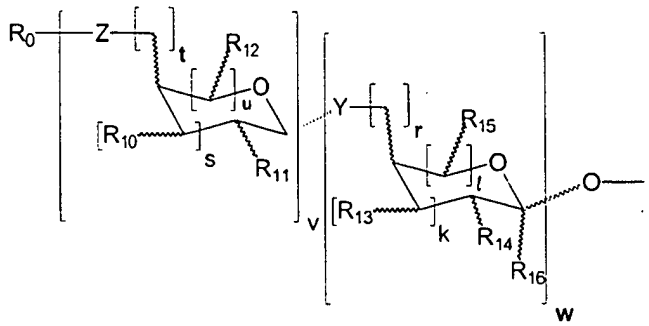
The present invention provides a glycoconjugate having the structure:

$$A\text{-}B_m\text{-}C_n\text{-}D_p\text{-}E_q\text{-}F$$

wherein m, n, p and q are 0, 1, 2 or 3 such that $m + n + p + q \leq 6$; wherein A, B, C, D, E and F are independently amino acyl or hydroxy acyl residues wherein A is N- or O-terminal and is either a free amine or ammonium form when A is amino acyl or a free hydroxy when A is hydroxy acyl, or A is alkylated, arylated or acylated; wherein F is either a free carboxylic acid, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester; wherein from one to about five of said amino acyl or hydroxy acyl residues are substituted by a carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3; wherein the carbohydrate domain is linked to the respective amino acyl or hydroxy acyl residue by substitution of a side group substituent selected from the group consisting of OH, COOH and NH_2; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:



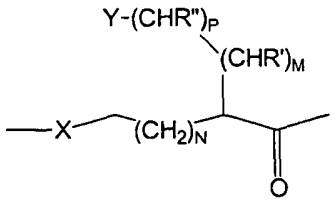
wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group.

In a certain embodiment, the present invention provides the glycoconjugate as shown above wherein at least one carbohydrate domain has the oligosaccharide structure of a cell surface epitope. In a particular embodiment, the

present invention provides the glycoconjugate wherein the epitope is Le^a, Le^b, Le^x, or Le^y. In another particular embodiment, the present invention provides the glycoconjugate wherein the epitope is MBr1, a truncated MBr1 pentasaccharide or a truncated MBr1 tetrasaccharide.

In another embodiment, the present invention provides a glycoconjugate wherein the amino acyl residue is derived from a natural amino acid. In another embodiment, the invention provides the glycoconjugate wherein at least one amino acyl residue has the formula: -NH-Ar-CO-. In a specific embodiment, the Ar moiety is p-phenylene.

In another embodiment, the present invention provides the glycoconjugate wherein at least one amino acyl or hydroxy acyl residue has the structure:



wherein M, N and P are independently 0, 1 or 2; X is NH or O; Y is OH, NH or COOH; and wherein R' and R'' are independently hydrogen, linear or branched chain alkyl or aryl. In a specific embodiment, the amino acyl residue attached to the carbohydrate domain is Ser or Thr.

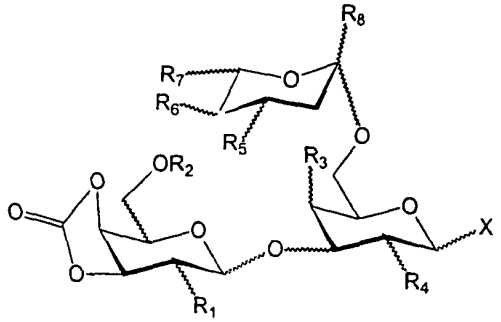
In another embodiment, the present invention provides the glycoconjugate wherein one or more of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8, R_9, R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is 1*RS*,2*RS*,3-trihydroxy-propyl.

The present invention also provides a pharmaceutical composition for treating cancer comprising the above-shown glycoconjugate and a pharmaceutically suitable carrier.

The present invention further provides a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of the above-shown glycoconjugate and a pharmaceutically suitable

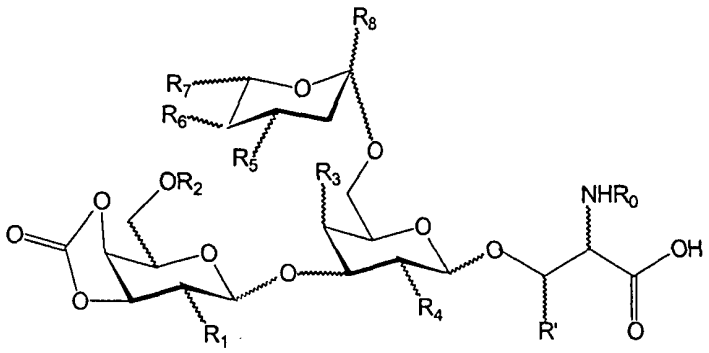
carrier. The method of treatment is effective when the cancer is a solid tumor or an epithelial cancer.

The present invention also provides a trisaccharide having the structure:



wherein R_1, R_3, R_4 , R_5, R_6 and R_7 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, N_3, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is H, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_2 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein R_8 is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein R^{ii} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein X is a halide, a trihaloacetamidate, an alkyl or aryl sulfide or a dialkylphosphite. In a preferred embodiment, the invention provides the above-shown trisaccharide wherein X is a triethylphosphite. The invention further provides the trisaccharide wherein R_7 is 1*RS*,2*RS*,3-trihydroxypropyl or 1*RS*,2*RS*,3-triacetoxypropyl. In addition, the invention provides the trisaccharide wherein R_8 is COOH.

The present invention also provides a trisaccharide amino acid having the structure:



wherein R_1, R_3, R_4 , R_5, R_6 and R_7 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, N_3, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is H, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_2 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein R_8 is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein R^{ii} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_0 is a base-labile N-protecting group; and wherein R' is hydrogen or a lower alkyl group. A variety of N-protecting groups would be acceptable in the preparation of the above-shown trisaccharide amino acid. R_0 may preferably be one of several base-sensitive protecting groups, but more preferably fluorenylmethyloxycarbonyl (FMOC).

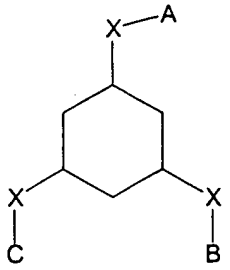
The present invention provides a method of inducing antibodies in a human subject, wherein the antibodies are capable of specifically binding with human tumor cells, which comprises administering to the subject an amount of the glycoconjugate disclosed herein effective to induce the antibodies. In a certain embodiment, the present invention provides a method of inducing antibodies wherein the glycoconjugate is bound to a suitable carrier protein. In particular, preferred examples of the carrier protein include bovine serum albumin, polylysine or KLH.

In another embodiment, the present invention contemplates a method of inducing antibodies which further comprises co-administering an immunological adjuvant. In a certain embodiment, the adjuvant is bacteria or liposomes. Specifically, favored

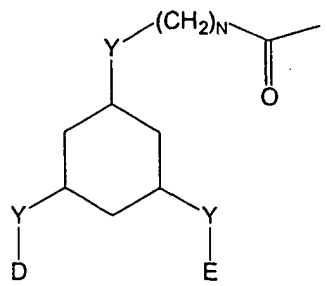
adjuvants include Salmonella minnesota cells, bacille Calmette-Guerin or QS21. The antibodies induced are typically selected from the group consisting of (2,6)-sialyl T antigen, Le^a, Le^b, Le^x, Le^y, GM1, SSEA-3 and MBrl antibodies. The method of inducing antibodies is useful in cases wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.

The present invention also provides a method of preventing recurrence of epithelial cancer in a subject which comprises vaccinating the subject with the glycoconjugate shown above which amount is effective to induce antibodies. In practicing this method, the glycoconjugate may be used alone or be bound to a suitable carrier protein. Specific examples of carrier protein used in the method include bovine serum albumin, polylysine or KLH. In a certain embodiment, the present method of preventing recurrence of epithelial cancer includes the additional step of co-administering an immunological adjuvant. In particular, the adjuvant is bacteria or liposomes. Favored adjuvants include *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21. The antibodies induced by the method are selected from the group consisting of (2,6)-sialyl T antigen, Le^a, Le^b, Le^x, Le^y, GM1, SSEA-3 and MBrl antibodies.

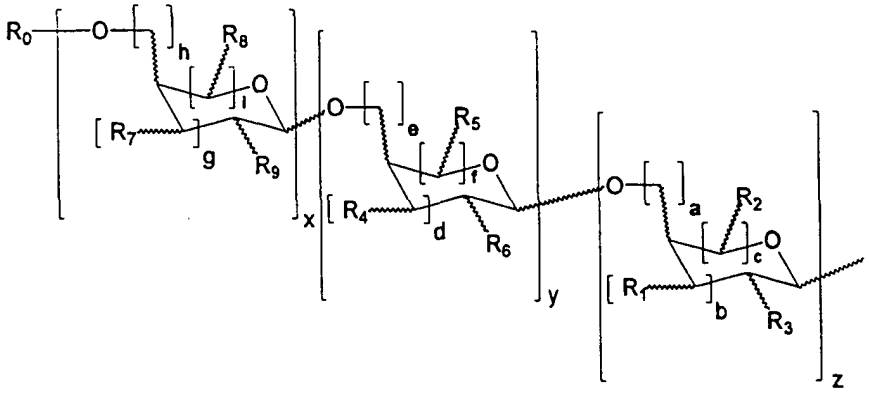
The present invention further provides a glycoconjugate having the structure:



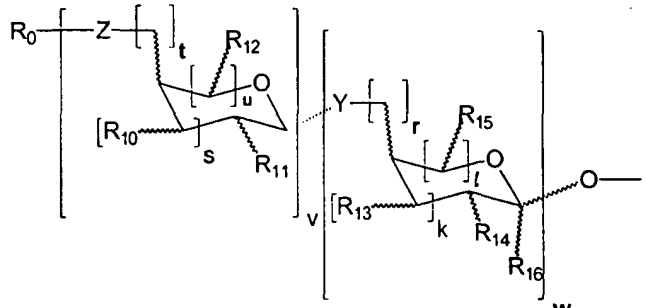
wherein X is O or NR; wherein R is H, linear or branched chain alkyl or acyl; wherein A, B and C independently linear or branched chain alkyl or acyl, $-CO-(CH_2)_P-OH$ or aryl, or have the structure:



wherein Y is O or NR; wherein D and E have the structure: $-(CH_2)_P-OH$ or $-CO-(CH_2)_P-OH$; wherein N and P are independently an integer between 0 and 12; wherein D and E and, when any of A, B and C are $-CO-(CH_2)_P-OH$, A, B and C are independently substituted by a carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3; wherein the carbohydrate domain is linked to the respective hydroxy acyl residue by substitution of a terminal OH substituent; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:

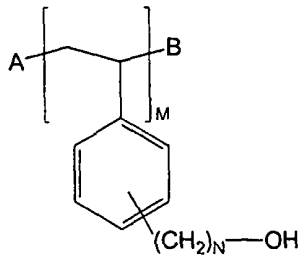


wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group. In a certain embodiment, the present invention provides the above-shown glycoconjugate wherein at least one carbohydrate domain has the oligosaccharide structure of a cell surface epitope. In one embodiment, the epitope is Le^a, Le^b, Le^x, or Le^y. In another embodiment, the epitope is MBr1, a truncated MBr1 pentasaccharide or a truncated MBr1 tetrasaccharide. In a particular embodiment, the invention provides the glycoconjugate shown above wherein one or more of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8, R_9, R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is 1*RS*,2*RS*,3-trihydroxy-propyl.

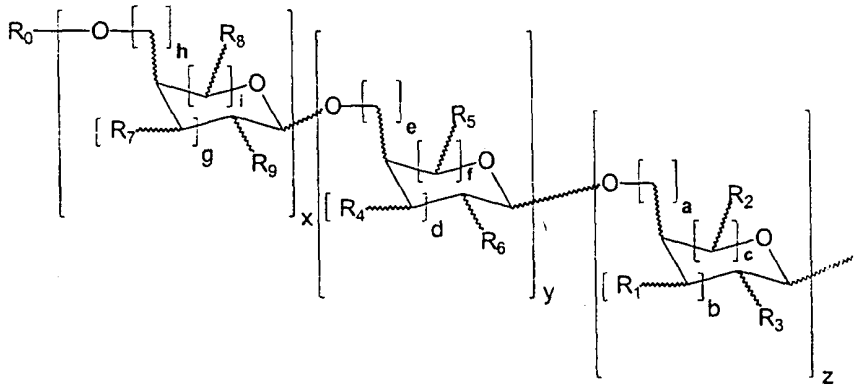
The invention also provides a pharmaceutical composition for treating cancer comprising the glycoconjugate shown above and a pharmaceutically suitable carrier.

The invention further provides a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of the glycoconjugate shown above and a pharmaceutically suitable carrier. The method is useful in cases where the cancer is a solid tumor or an epithelial cancer.

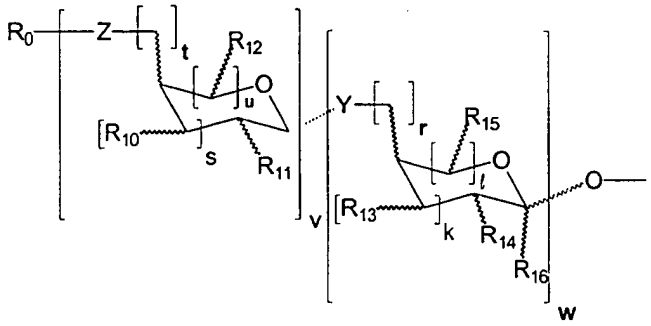
The present invention also provides a glycoconjugate comprising a core structure and a carbohydrate domain wherein the core structure is:



wherein M is an integer from about 2 to about 5,000; wherein N is 1, 2, 3 or 4; wherein A and B are suitable polymer termination groups, including linear or branch chain alkyl or aryl groups; wherein the core structure is substituted by the carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3; wherein the carbohydrate domain is linked to the core structure by substitution of the OH substituents; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group.

In a specific embodiment, the present invention provides a method of preparing glycopeptides related to the mucin family of cell surface glycoproteins. Mucins are characterized by aberrant α-O-glycosidation patterns with clustered arrangements of carbohydrates α-O-linked to serine and threonine residues. Figure 1. Mucins are common markers of epithelial tumors (*e.g.*, prostate and breast carcinomas) and certain blood cell tumors. Finn, O.J., *et al.*, *Immunol. Rev.* **1995**, *145*, 61.

The (2,6)-Sialyl T antigen (ST antigen) is an example of the "glycophorin family" of α-O-linked glycopeptides (Figure 2). It is selectively expressed on myelogenous leukemia cells. Fukuda, M., *et al.*, *J. Biol. Chem.* **1986**, *261*, 12796. Saitoh, O., *et al.*, *Cancer Res.* **1991**, *51*, 2854. Thus, in a specific embodiment, the present invention provides a synthetic route to pentapeptide **1**, which is derived from the N-terminus of CD43 (Leukosialin) glycoprotein. Pallant, A., *et al.*, *Proc. Natl. Acad. Sci.*

USA **1989**, *86*, 1328.

In particular, the invention provides a stereoselective preparation of α-O-linked (2,6)-ST glycosyl serine and threonine *via* a block approach. In addition, the present invention provides an O-linked glycopeptide incorporating such glycosyl units with clustered ST epitopes **(1,20)**.

A broad range of carbohydrate domains are contemplated by the present invention. Special mention is made of the carbohydrate domains derived from the following cell surface epitopes and antigens:

MBr1 Epitope: Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glu-0cer

Truncated MBr1 Epitope Pentasaccharide:

Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1

Truncated MBr1 Epitope Tetrasaccharide: Fucα1-2Galβ1-3GalNAcβ1-3Galα1

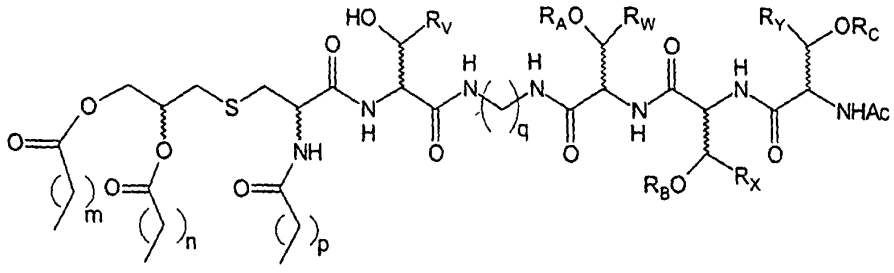
SSEA-3 Antigen: 2Galβ1-3GalNAcβ1-3Galα1-4Galβ1

Le^y Epitope: Fucα1-2Galβ1-4(Fucα1-3)GalNAcβ1

GM1 Epitope: Galβ1-3GalNAcβ1-4Galβ1-4(NeuAcα2-3)Glu-0cer

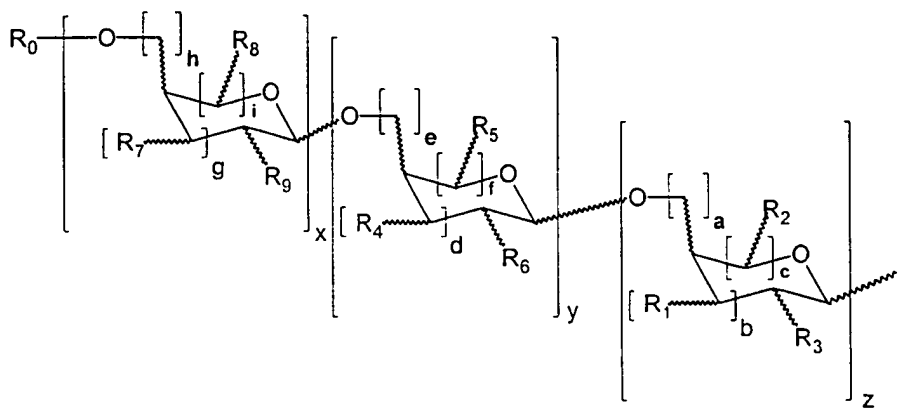
Methods for preparing carbohydrate domains based on a solid-phase methodology have been disclosed in U.S. Serial Nos. 08/213,053 and 08/430,355, and in PCT International Application No. PCT/US96/10229, the contents of which are incorporated by reference.

The present invention also provides a glycoconjugate having the structure:

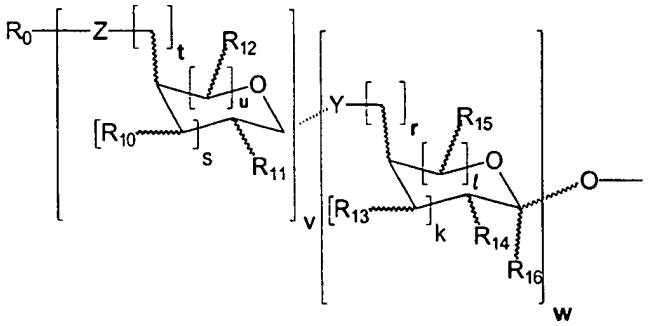


wherein *m*, *n* and *p* are integers between about 8 and about 20; wherein *q* is an integer

between about 1 and about 8; wherein R_V, R_W, R_X and R_Y are independently hydrogen, optionally substituted linear or branched chain lower alkyl or optionally substituted phenyl; wherein R_A, R_B and R_C are independently a carbohydrate domain having the structure:



wherein *a, b, c, d, e, f, g, h, i, x, y* and *z* are independently 0, 1, 2 or 3; wherein R_0 is hydrogen, linear or branched chain lower alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:

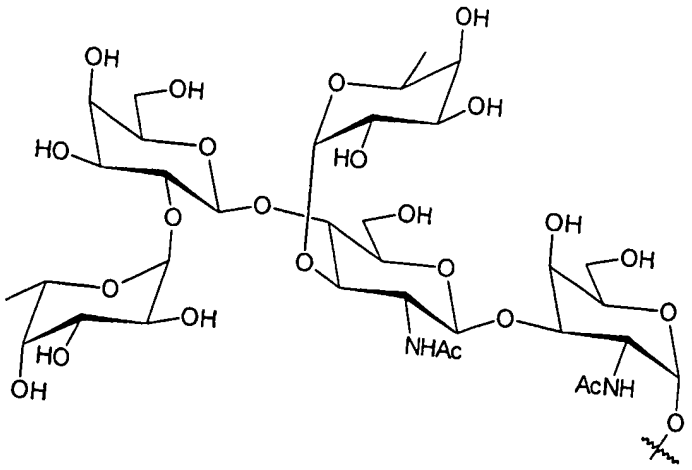


wherein Y and Z are independently NH or O; wherein *k, l, r, s, t, u, v* and *w* are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or an optionally substituted

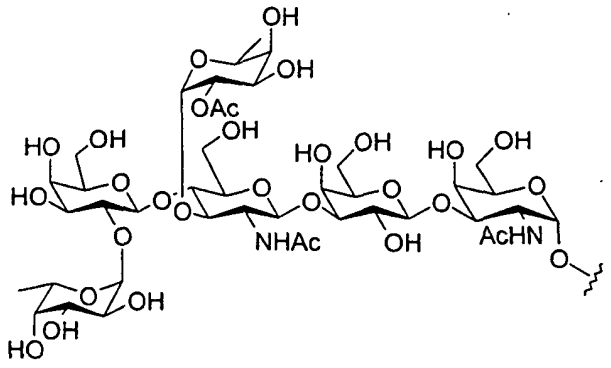
linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, optionally substituted linear or branched chain lower alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently hydrogen, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group. In a certain embodiment, the invention provides a glycoconjugate wherein R_v, R_w, R_x and R_y are methyl.

In a certain other embodiment, the carbohydrate domains may be independently monosaccharides or disaccharides. In one embodiment, the invention provides a glycoconjugate wherein *y* and *z* are 0; wherein *x* is 1; and wherein R_3 is NHAc. In another embodiment, the invention provides a glycoconjugate wherein *h* is 0; wherein *g* and *i* are 1; wherein R_7 is OH; wherein R_9 is hydrogen; and wherein R_8 is hydroxymethyl. In yet another embodiment, *m*, *n* and *p* are 14; and wherein q is 3. In a preferred embodiment, each amino acyl residue of the glycoconjugate therein has an L-configuration.

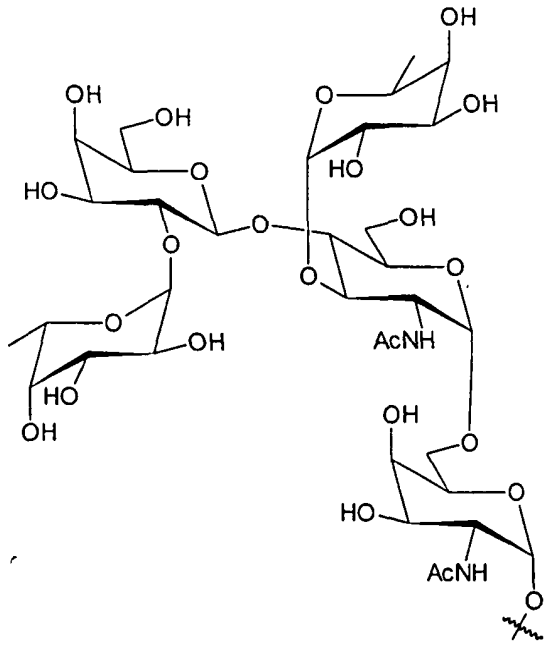
In a specific example, the carbohydrate domains of the glcyoconjugate are independently:



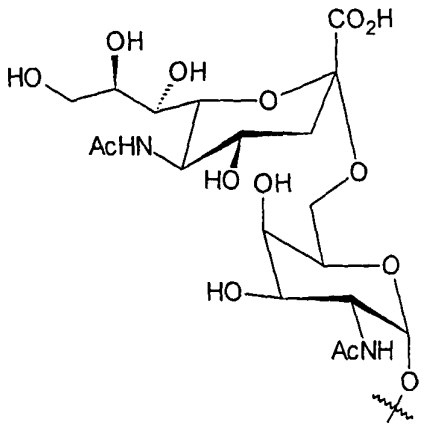
In another example, the carbohydrate domains are independently:



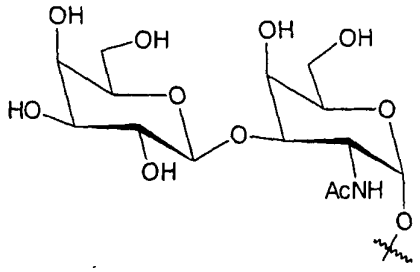
In another example, the carbohydrate domains are independently:



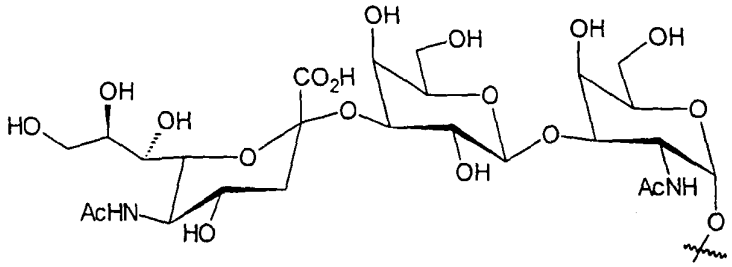
Additionally, the carbohydrate domains are independently:



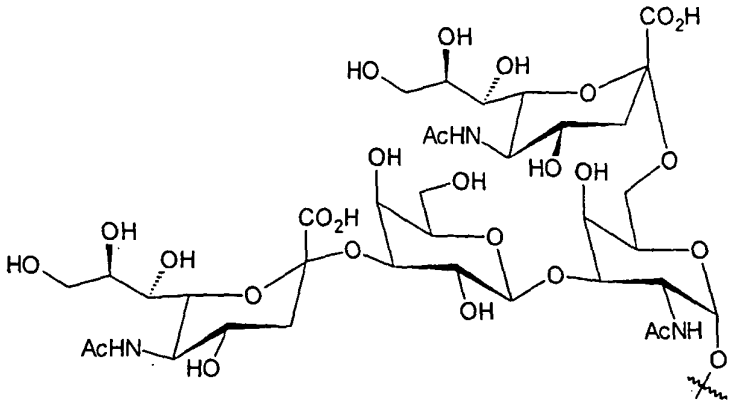
The carbohydrate domains are also independently:



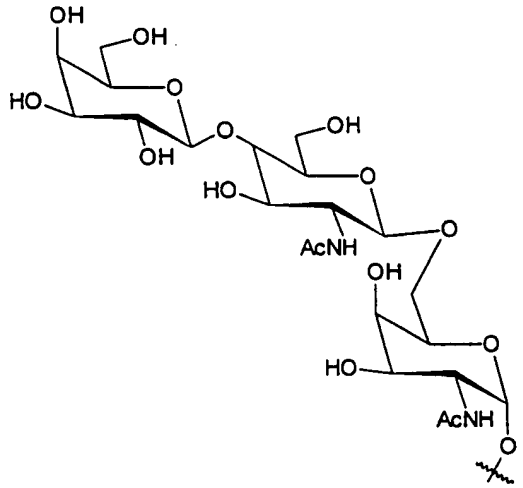
The carbohydrate domains also are independently



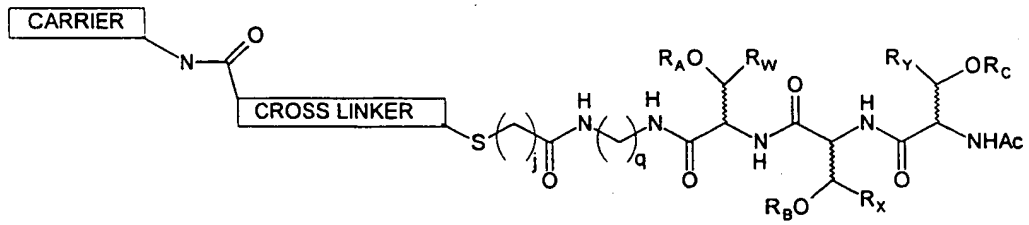
Also, the carbohydrate domains may be independently:



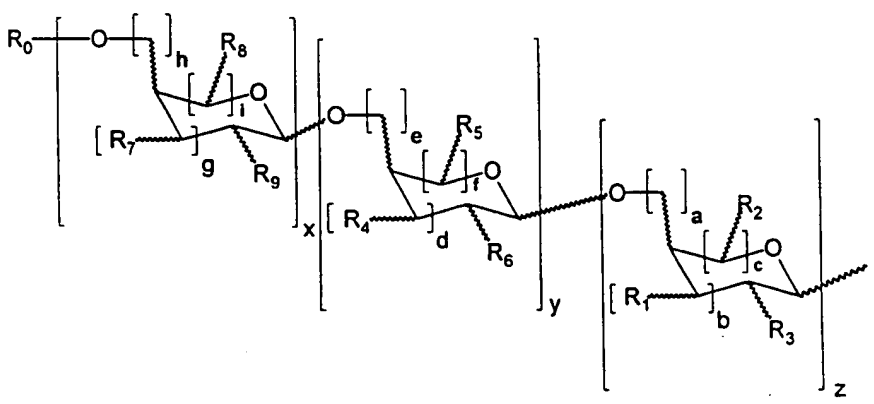
The carbohydrate domains are also independently:



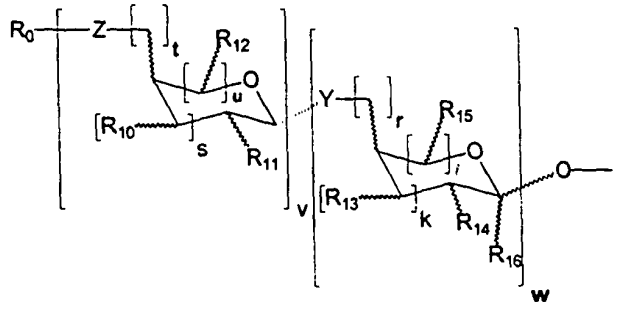
The present invention provides a glycoconjugate having the structure:



wherein the carrier is a protein; wherein the cross linker is a moiety derived from a cross linking reagent capable of conjugating a surface amine of the carrier and a thiol; wherein *m*, *n* and *p* are integers between about 8 and about 20; wherein *j* and *q* are independently integers between about 1 and about 8; wherein R_V, R_W, R_X and R_Y are independently hydrogen, optionally substituted linear or branched chain lower alkyl or optionally substituted phenyl; wherein R_A, R_B and R_C are independently a carbohydrate domain having the structure:



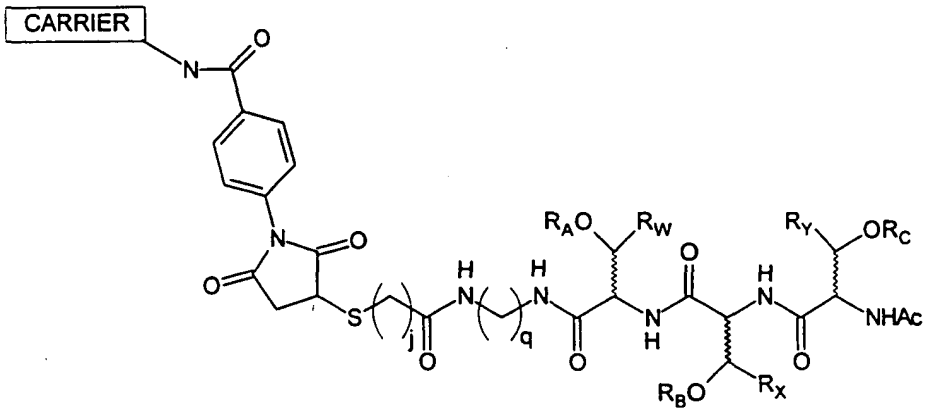
wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3; wherein R_0 is hydrogen, linear or branched chain lower alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein *k*, *l*, *r*, *s*, *t*, *u*, *v* and *w* are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, optionally substituted linear or branched chain lower alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently hydrogen, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group.

Various proteins are contemplated as being suitable, including bovine serum albumin, KLH, and human serum albumin. Cross linkers suited to the invention are

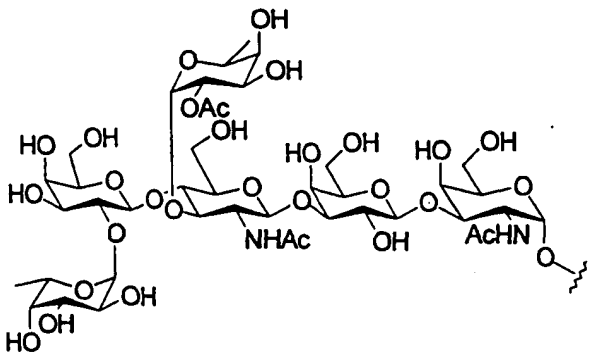
widely known in the art, including bromoacetic NHS ester, 6-(iodoacetamido)caproic acid NHS ester, maleimidoacetic acid NHS ester, maleimidobenzoic acid NHS ester, etc., In one embodiment, the glycoconjugate has the structure:



In one embodiment, the invention provides the glycoconjugate wherein R_v, R_w, R_x and R_y are methyl. In another embodiment, the invention provides the glycoconjugate wherein the carbohydrate domains are monosaccharides or disaccharides. In another embodiment, the invention provides the glycoconjugate wherein *y* and *z* are 0; wherein *x* is 1; and wherein R_3 is NHAc. In a further embodiment, the invention provides the glycoconjugate wherein *h* is 0; wherein *g* and *i* are 1; wherein R_7 is OH; wherein R_0 is hydrogen; wherein *m*, *n* and *p* are 14; and wherein q is 3; and wherein R_8 is hydroxymethyl.

In a certain embodiment, the invention provides the glycoconjugate as disclosed wherein the protein is BSA or KLH. In a preferred embodiment, each amino acyl residue of the glycoconjugate has an L-configuration.

Specific examples of the glycoconjugate contain any of the following carbohydrate domains, which may be either the same or different in any embodiment.



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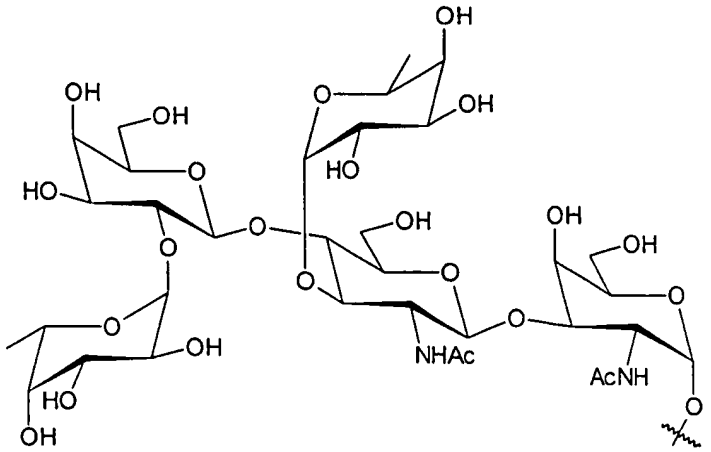
OH
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OH
HO
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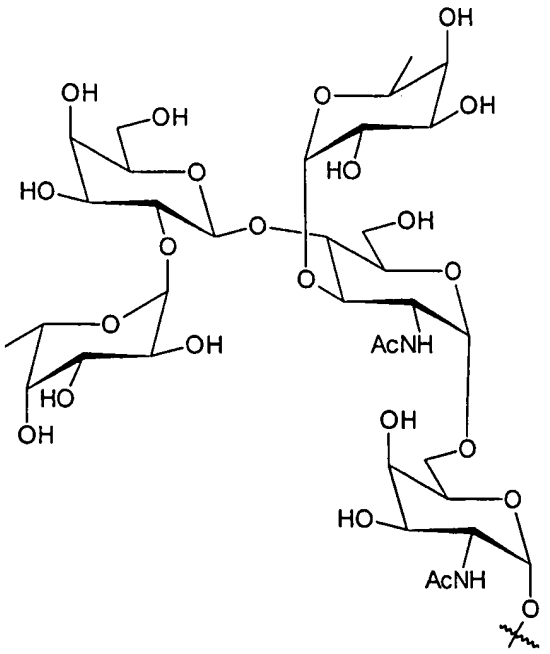
NHAc
OH
OH
O
AcNH
O


OH
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AcNH
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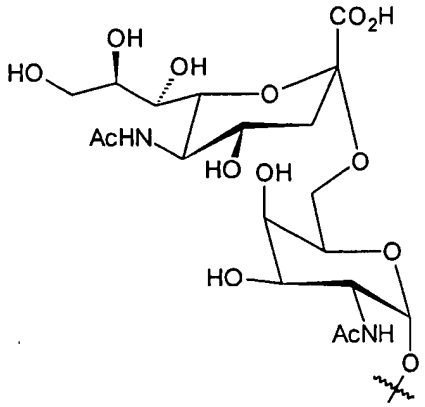
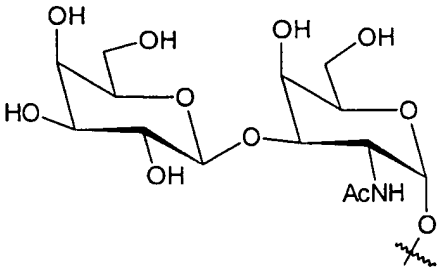
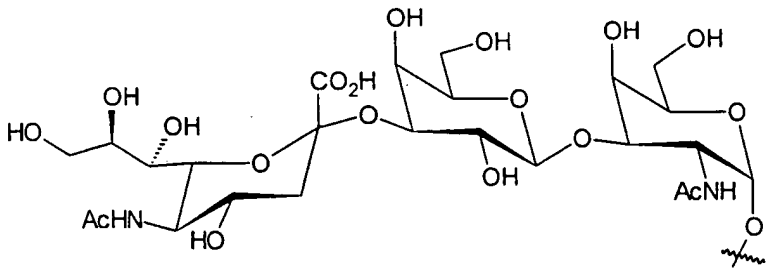
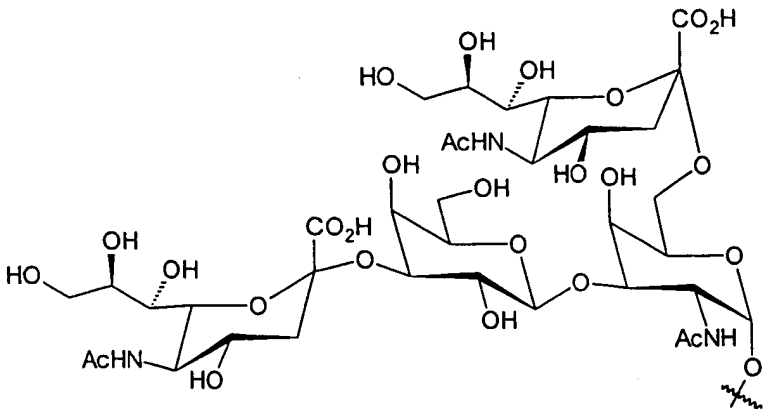
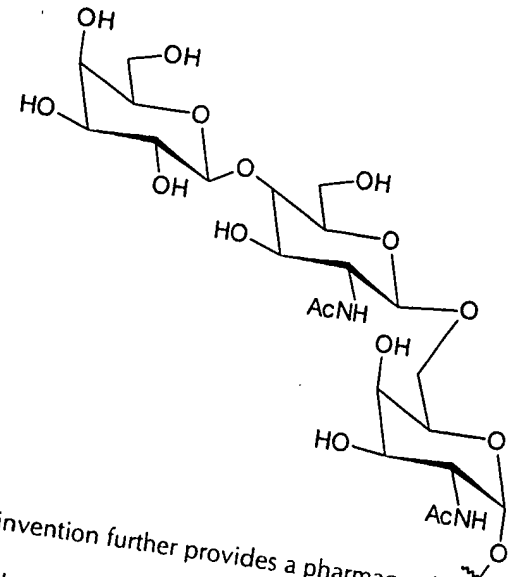










The present invention further provides a pharmaceutical composition for treating cancer comprising a glycoconjugate as above disclosed and a pharmaceutically suitable carrier.

The invention also provides a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of a glycoconjugate disclosed above and a pharmaceutically suitable carrier. In a certain embodiment, the invention provides the method wherein the cancer is a solid tumor. Specifically, the method is applicable wherein the cancer is an epithelial cancer. Particularly effective is the application to treat prostate cancer.

The invention also provides a method of inducing antibodies in a human subject, wherein the antibodies are capable of specifically binding with human tumor cells, which comprises administering to the subject an amount of the glycoconjugate disclosed above effective to induce the antibodies. In a certain embodiment, the invention provides the method wherein the carrier protein is bovine serum albumin, polylysine or KLH.

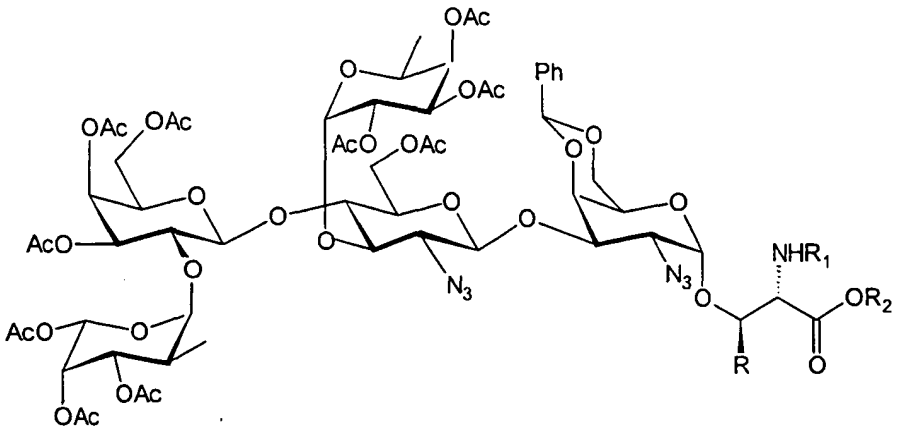
In addition, the invention provides the related method of inducing antibodies which further comprises co-administering an immunological adjuvant. The adjuvant is preferably bacteria or liposomes. In particular, the adjuvant is *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21. The antibodies induced are favorably

selected from the group consisting of Tn, ST_N, (2,3)ST, glycophorine, 3-Le^y, 6-Le^y, T(TF) and T antibodies.

The invention further provides the method of inducing antibodies wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.

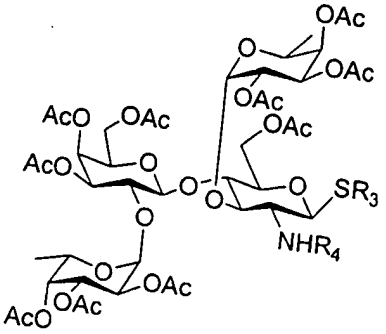
The invention also provides a method of preventing recurrence of epithelial cancer in a subject which comprises vaccinating the subject with the glycoconjugate disclosed above which amount is effective to induce antibodies. The method may be practiced wherein the carrier protein is bovine serum albumin, polylysine or KLH. In addition, the invention provides the related method of preventing recurrence of epithelial cancer which further comprises co-administering an immunological adjuvant. Preferably, the adjuvant is bacteria or liposomes. Specifically, the preferred adjuvant is *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21. The antibodies induced in the practice of the methods are selected from the group consisting of Tn, ST_N, (2,3)ST, glycophorine, 3-Le^y, 6-Le^y, T(TF) and T antibodies.

The present invention also provides a method of preparing a protected O-linked Le^y glycoconjugate having the structure:

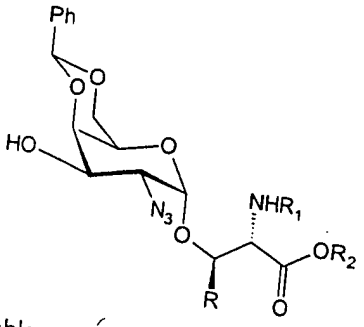


wherein R is hydrogen, linear or branched chain lower alkyl, or optionally substituted aryl; R_1 is t-butyloxycarbonyl, fluorenylmethyleneoxycarbonyl, linear or branched chain lower alkyl or acyl, optionally substituted benzyl or aryl; R_2 is a linear or branched chain lower

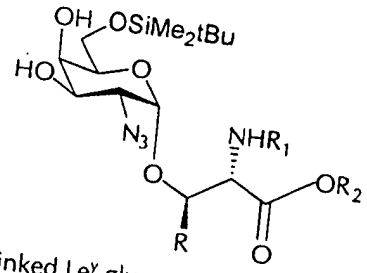
alkyl, or optionally substituted benzyl or aryl; and R_4 is hydrogen, linear or branched chain lower alkyl or acyl, optionally substituted aryl or benzyl, or optionally substituted aryl sulfonyl; which comprises coupling a tetrasaccharide sulfide having the structure:



wherein R_3 is linear or branched chain lower alkyl or aryl; with an O-linked glycosyl amino acyl component having the structure:

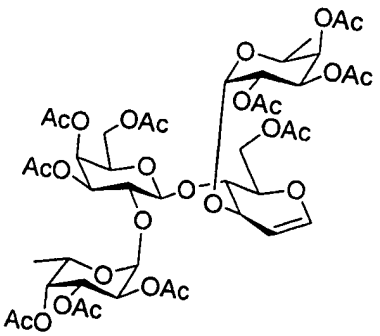


or



under suitable conditions to form the protected O-linked Le^y glycoconjugate.

In one embodiment of the invention, the tetrasaccharide sulfide shown above may be prepared by (a) halosulfonamidating a tetrasaccharide glycal having the structure:

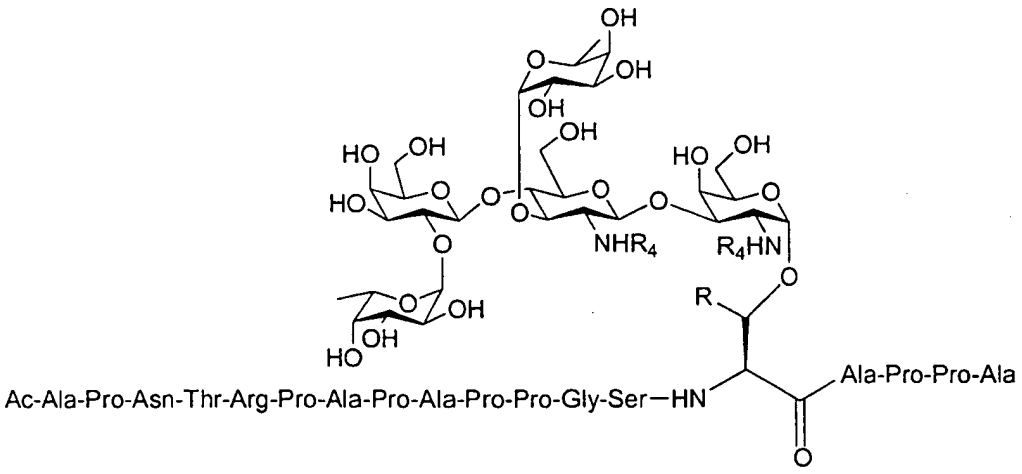


under suitable conditions to form a tetrasaccharide halosulfonamidate; and

(b) treating the halosulfonamidate with a mercaptan and a suitable base to form the tetrasaccharide sulfide. In particular, the method may be practiced wherein the mercaptan is a linear or branched chain lower alkyl or an aryl; and the base is sodium hydride, lithium hydride, potassium hydride, lithium diethylamide, lithium diisopropylamide, sodium amide, or lithium hexamethyldisilazide.

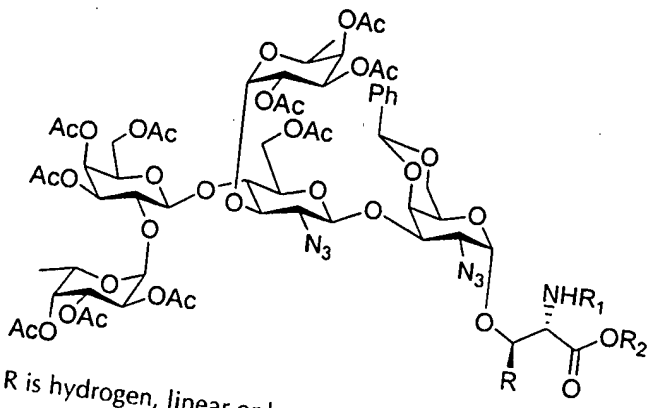
The invention also provides an O-linked glycoconjugate prepared by the method disclosed.

In particular, the invention provides an O-linked glycopeptide having the structure:

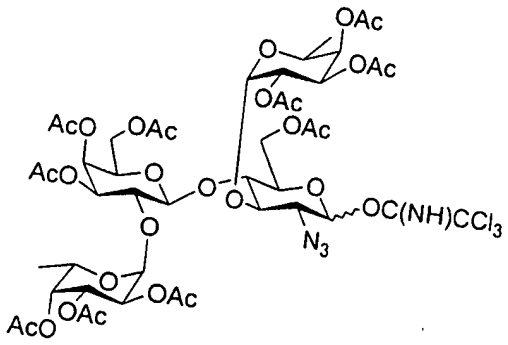


wherein R_4 is a linear or branched chain lower acyl; and wherein R is hydrogen or a linear or branched chain lower alkyl or aryl. Variations in the peptidic portion of the glycopeptide are within the scope the invention. In a specific embodiment, the invention provides the O-linked glycopeptide wherein R_4 is acetyl.

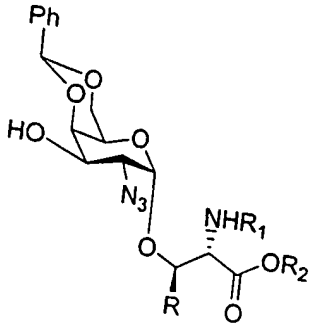
The present invention provides a method of preparing a protected O-linked Le^y glycoconjugate having the structure:



wherein R is hydrogen, linear or branched chain lower alkyl, or optionally substituted aryl; R_1 is t-butyloxycarbonyl, fluorenylmethyleneoxycarbonyl, linear or branched chain lower alkyl or acyl, optionally substituted benzyl or aryl; and R_2 is a linear or branched chain lower alkyl, or optionally substituted benzyl or aryl; which comprises coupling a tetrasaccharide azidoimidate having the structure:

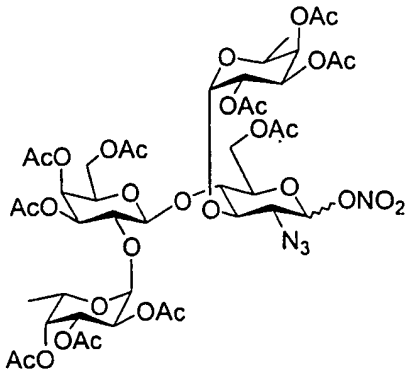


with an O-linked glycosyl amino acyl component having the structure:

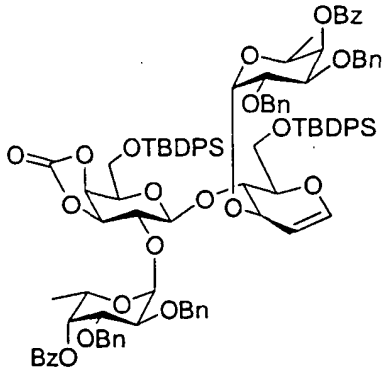


under suitable conditions to form the protected O-linked Le^y glycoconjugate. The tetrasaccharide azidoimidate is favorably prepared by (a) treating tetrasaccharide

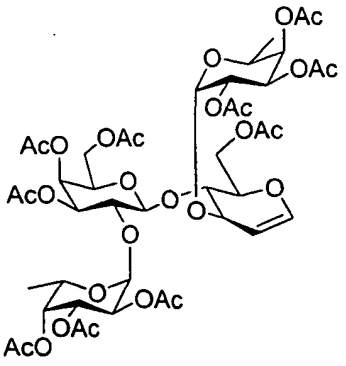
azidonitrate having the structure:



under suitable conditions to form an azido alcohol; and (b) reacting the azido alcohol with an imidoacylating reagent under suitable conditions to form the azidoimidate. The tetrasaccharide azido nitrate may be prepared by (a) converting a tetrasaccharide glycal having the structure:



under suitable conditions to a peracetylated tetrasaccharide glycal having the structure:



and (b) azidonitrating the glycal formed in step (a) under suitable conditions to form the tetrasaccharide azido nitrate. Step (b) is favorably effected using cerium ammonium nitrate in the presence of an azide salt selected from the group consisting of sodium azide, lithium azide, potassium azide, tetramethylammonium azide and tetraethylammonium azide.

In addition, the invention provides an O-linked glycoconjugate prepared as shown above.

Once the carbohydrate domains covalently linked to O-bearing aminoacyl side chains are prepared, the glycoconjugates of the subject invention may be prepared using either solution-phase or solid-phase synthesis protocols, both of which are well-known in the art for synthesizing simple peptides. Among other methods, a widely used solution phase peptide synthesis method useful in the present invention uses FMOC (or a related carbamate) as the protecting group for the α-amino functional group; ammonia, a primary or secondary amine (such as morpholine) to remove the FMOC protecting group and a substituted carbodiimide (such as N,N'-dicyclohexyl- or -diisopropylcarbodiimide) as the coupling agent for the C to N synthesis of peptides or peptide derivatives in a proper organic solvent. Solution-phase and solid phase synthesis of O-linked glycoconjugates in the N to C direction is also within the scope of the subject invention.

For solid-phase synthesis, several different resin supports have been adopted as standards in the field. Besides the original chloromethylated polystyrene of Merrifield, other types of resin have been widely used to prepare peptide amides and acids, including benzhydrylamine and hydroxymethyl resins (Stewart, *Solid Phase Peptide*

Synthesis, Pierce Chemical Co., **1984**, Rockford, IL; Pietta, *et al., J. Chem. Soc.* D., **1970**, 650-651; Orlowski, *et al, J. Org. Chem.*, **1976**, *50,* 3701-5; Matsueda et al, Peptides, **1981**, *2,* 45-50; and Tam, *J. Org. Chem.*, **1985**, *50,* 5291-8) and a resin consisting of a functionalized polystyrene-grafted polymer substrate (U.S. Patent No. 5,258,454). These solid phases are acid labile (Albericio, *et al., Int. J. Peptide Research.* **1987**, *30,* 206-216). Another acid labile resin readily applicable in practicing the present invention uses a trialkoxydi-phenylmethylester moiety in conjunction with FMOC-protected amino acids (Rink, *Tetrahedron Letters,* **1987**, *28,* 3787-90; U.S. Pat. No. 4,859,736; and U.S. Pat. No. 5,004,781). The peptide is eventually released by cleavage with trifluoroacetic acid. Adaptation of the methods of the invention for a particular resin protocol, whether based on acid-labile or base-sensitive N-protecting groups, includes the selection of compatible protecting groups, and is within the skill of the ordinary worker in the chemical arts.

The glycoconjugates prepared as disclosed herein are useful in the treatment and prevention of various forms of cancer. Thus, the invention provides a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of any of the α-O-linked glycoconjugates disclosed herein, optionally in combination with a pharmaceutically suitable carrier. The method may be applied where the cancer is a solid tumor or an epithelial tumor, or leukemia. In particular, the method is applicable where the cancer is breast cancer, where the relevant epitope may be MBr1.

The subject invention also provides a pharmaceutical composition for treating cancer comprising any of the α-O-linked glycoconjugates disclosed hereinabove, as an active ingredient, optionally though typically in combination with a pharmaceutically suitable carrier. The pharmaceutical compositions of the present invention may further comprise other therapeutically active ingredients.

The subject invention further provides a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of any of the α-O-linked glycoconjugates disclosed hereinabove and a

pharmaceutically suitable carrier.

The compounds taught above which are related to α-O-linked glycoconjugates are useful in the treatment of cancer, both *in vivo* and *in vitro*. The ability of these compounds to inhibit cancer cell propagation and reduce tumor size in tissue culture, as demonstrated in the accompanying data tables, will show that the compounds are useful to treat, prevent or ameliorate cancer in subjects suffering therefrom.

In addition, the glycoconjugates prepared by processes disclosed herein are antigens useful in adjuvant therapies as vaccines capable of inducing antibodies immunoreactive with various epithelial tumor and leukemia cells. Such adjuvant therapies may reduce the rate of recurrence of epithelial cancers and leukemia, and increase survival rates after surgery. Clinical trials on patients surgically treated for cancer who are then treated with vaccines prepared from a cell surface differentiation antigen found in patients lacking the antibody prior to immunization, a highly significant increase in disease-free interval may be observed. Cf. P.O. Livingston, et al., *J. Clin. Oncol.*, **1994**, 12, 1036.

The magnitude of the therapeutic dose of the compounds of the invention will vary with the nature and severity of the condition to be treated and with the particular compound and its route of administration. In general, the daily dose range for anticancer activity lies in the range of 0.001 to 25 mg/kg of body weight in a mammal, preferably 0.001 to 10 mg/kg, and most preferably 0.001 to 1.0 mg/kg, in single or multiple doses. In unusual cases, it may be necessary to administer doses above 25 mg/kg.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound disclosed herein. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, etc., routes may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, etc.

The compositions include compositions suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions and dusting powders),

parenteral (including subcutaneous, intramuscular and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation) or nasal administration. Although the most suitable route in any given case will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

In preparing oral dosage forms, any of the unusual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (e.g., suspensions, elixers and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, etc., in the case of oral solid preparations are preferred over liquid oral preparations such as powders, capsules and tablets. If desired, capsules may be coated by standard aqueous or non-aqueous techniques. In addition to the dosage forms described above, the compounds of the invention may be administered by controlled release means and devices.

Pharmaceutical compositions of the present invention suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient in powder or granular form or as a solution or suspension in an aqueous or nonaqueous liquid or in an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any of the methods known in the art of pharmacy. In general compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers, finely divided solid carriers, or both and then, if necessary, shaping the product into the desired form. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granule optionally mixed with a binder, lubricant, inert diluent or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound

moistened with an inert liquid diluent.

The present invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described in the claims which follow thereafter. It will be understood that the processes of the present invention for preparing α-O-linked glycoconjugates encompass the use of various alternate protecting groups known in the art. Those protecting groups used in the disclosure including the Examples below are merely illustrative.

Experimental Details: General Procedures

All air- and moisture-sensitive reactions were performed in a flame-dried apparatus under an argon atmosphere unless otherwise noted. Air-sensitive liquids and solutions were transferred via syringe or canula. Wherever possible, reactions were monitored by thin-layer chromatography (TLC). Gross solvent removal was performed in vacuum under aspirator vacuum on a Buchi rotary evaporator, and trace solvent was removed on a high vacuum pump at 0.1-0.5 mmHg.

Melting points (mp) were uncorrected and performed in soft glass capillary tubes using an Electrothermal series IA9100 digital melting point apparatus. Infrared spectra (IR) were recorded using a Perkin-Elmer 1600 series Fourier-Transform instrument. Samples were prepared as neat films on NaCl plates unless otherwise noted. Absorption bands are reported in wavenumbers (cm^{-1}). Only relevant, assignable bands are reported.

Proton nuclear magnetic resonance (^1H NMR) spectra were determined using a Bruker AMX-400 spectrometer at 400 MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS; $\delta = 0$ ppm) using residual $CHCl_3$ as a lock reference ($\delta = 7.25$ ppm). Multiplicities are abbreviated in the usual fashion: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were performed on a Bruker AMX-400

spectrometer at 100 MHz with composite pulse decoupling. Samples were prepared as with ^1H NMR spectra, and chemical shifts are reported relative to TMS (0 ppm) ; residual $CHCl_3$ was used as an internal reference ($\delta = 77.0$ ppm). All high resolution mass spectral (HRMS) analyses were determined by electron impact ionization (EI) on a JEOL JMS-DX 303HF mass spectrometer with perfluorokerosene (PFK) as an internal standard. Low resolution mass spectra (MS) were deter-mined by either electron impact ionization (EI) or chemical ionization (CI) using the indicated carrier gas (ammonia or methane) on a Delsi–Nermag R-10-10 mass spectrometer. For gas chromatography/mass spectra (GCMS), a DB-5 fused capillary column (30 m, 0.25mm thickness) was used with helium as the carrier gas. Typical conditions used a temperature program from 60-250°C at 40°C/min.

Thin layer chromatography (TLC) was performed using precoated glass plates (silica gel 60, 0.25 mm thickness). Visualization was done by illumination with a 254 nm UV lamp, or by immersion in anisaldehyde stain (9.2 mL p-anisaldehyde in 3.5 mL acetic acid, 12.5 mL conc. sulfuric acid and 338 mL 95.% ethanol (EtOH)) and heating to colorization. Flash silica gel chromatography was carried out according to the standard protocol.

Unless otherwise noted, all solvents and reagents were commercial grade and were used as received, except as indicated hereinbelow, where solvents were distilled under argon using the drying methods listed in parentheses: CH_2Cl_2 (CaH_2); benzene (CaH_2); THF (Na/ketyl); Et_2O (Na/ketyl); diisopropylamine (CaH_2).

Abbreviations

TLC	thin layer chromatography
EtOAc	ethyl acetate
TIPS	triisopropylsilyl
PMB	p-methoxybenzyl
Bn	benzyl
Ac	acetate

hex	hexane
THF	tetrahydrofuran
coll	collidine
LiHMDS	lithium hexamethyldisilazide
DMF	N,N-dimethylformamide
DMAP	2-dimethylaminopyridine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
TBAF	tetra-n-butylammonium fluoride
M.S.	molecular sieves
r.t.	room temperature
r.b.	round bottom flask

EXAMPLE 1

2,6-Di-O-acetyl-3,4-O-carbonyl-β-D-galactopyranosyl-(1-3)-6-O-(triisopropylsilyl)-4-O-acetyl-galactal (3). Galactal **2** (1.959g, 9.89 mmol, 1.2 eq.) was dissolved in 100 mL of anhydrous CH_2Cl_2 and cooled to 0°C. Solution of dimethyldioxirane (200 mL of ca 0.06M solution in acetone) was added *via* cannula to the reaction flask. After 1 hr the starting material was consumed as judged by TLC. Solvent was removed with a stream of N_2 and the crude epoxide was dried *in vacuo* for 1 hr at room temperature. The crude residue (single spot by TLC) was taken up in 33mL of THF and 6-O-triisopropyl-galactal acceptor (2.50g, 8.24 mmol) in 20 mL THF was added. The resulting mixture was cooled to -78°C and $ZnCl_2$ (9.8mL of 1M solution in ether) was added dropwise. The reaction was slowly warmed up to rt and stirred overnight. The mixture was diluted with EtOAc and washed with sat. sodium bicarbonate, then with brine and finally dried over $MgSO_4$. After evaporation of the solvent the crude material was purified by flash chromatography (40-45-50-60% EtOAc/hexane) to yield pure product which was immediately acetylated. 3.36g was dissolved in 50 mL of dry CH_2Cl_2, triethylamine (19.2 mL), cat amount of DMAP (ca 20mg) were added and the solution was cooled to 0C. Acetic anhydride (9.9 mL) was

added dropwise at 0°C. The reaction was stirred at rt overnight. The solvent was removed *in vacuo* and the crude material was chromatographed (50% EtOAc/hexane) to give glycal **3** (3.3g, 75%): ^{1}H NMR (500MHz, $CDCl_3$) δ 6.42 (d, J = 6.3 Hz, 1H, H-1, glycal), 4.35 (½ AB, dd, J = 6.8 Hz, 11.5 Hz, 1H, H-6'a), 4.28 (1/2AB, dd, J = 6.1, 11.5 Hz, 1H, H-6'b).

EXAMPLE 2

2,6-Di-O-acetyl-3,4-O-carbonyl-β-D-galactopyranosyl-(1-3)-4-O-acetyl-galactal (4). Compound **3** (1.5 g, 2.43 mmol) was dissolved in 24 mL of THF and cooled to 0°C. A mixture of TBAF (5.8 mL, 5.83 mmol, 2.4 eq.) and acetic acid (336 mL, 2.4 eq.) was added to the substrate at 0°C. The reaction was stirred at 30°C for 5 hrs. The reaction mixture was diluted with ethyl acetate and quenched with sat sodium bicarbonate. Organic phase was washed with sat sodium bicarbonate, brine and subsequently dried over magnesium sulphate. The crude product was purified by chromatography (80-85-90% EtOAc/ hexane) to yield compound **4** (0.9 g, 80%): ^{1}H NMR (500MHz, $CDCl_3$) δ 6.38 (dd, J = 1.8, 6.3 Hz, 1H, H-1, glycal), 5.39 (m, 1H, H-4), 2.22 (s, 3H, acetate), 2.16 (s, 3H, acetate), 2.13 (s, 3H, acetate).

EXAMPLE 3

[(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-O-glycero-α-D-galacto-2-nonulopyranosylonate)-(2-6)]-(2,6-di-O-acetyl-3,4-O-carbonyl-β-D-galactopyranosyl)-(1-3)-4-O-acetyl-galactal. (6). A flame dried flask was charged with sialyl phosphite donor **5** (69 mg, 0.11 mmol, 1.3 eq.) and acceptor **4** (40 mg, 0.085 mmol) in the dry box (Argon atmosphere). The mixture was dissolved in 0.6 mL of dry THF. 0.6 mL of dry toluene was added and the solution was slowly cooled to -60°C to avoid precipitation. Trimethylsilyl triflate (2.4 μL, 0.11 eq.) was added and the mixture was stirred at -45°C. The reaction was quenched at -45°C after 2 hrs (completion judged by TLC) with 2 mL of sat. sodium bicarbonate, warmed until water melted and the mixture was poured into an excess of

ethyl acetate. Organic layer was washed with sat. sodium bicarbonate and dried over anhydrous sodium sulphate. ^{1}H NMR of the crude material revealed a 4:1 ratio of α:β isomers (66.4 mg, 84%). The mixture was separated by flash chromatography on silica gel (2-2.5-3-3.5-4% MeOH/CH_2Cl_2) to yield compound **6** (50 mg, 63 % yield): ^{1}H NMR (500MHz, $CDCl_3$) δ 6.42 (d, J = 6.2 Hz, 1H), 5.37 (m, 1H), 5.32 - 5.29 (m, 4H), 5.26 - 5.24 (m, 1H), 5.12 - 5.10 (m, 2H), 4.98 (d, J = 3.5 Hz, 1H), 4.92 - 4.85 (m, 1H), 4.83 - 4.80 (m, 3H), 4.54 (m, 1H), 4.45 (dd, J = 3.0, 13.5 Hz, 1H), 4.33 - 4.20 (m, 3H), 4.22 - 4.02 (m, 7H), 3.96 (dd, J = 7.6, 10.9 Hz, 1H, H-2), 2.59 (dd, J = 4.6, 12.9 Hz, 1H, H-2e NeuNAc), 2.30 (dd, J = 12.9 Hz, 1H, H-2ax NeuNAc), 2.16, 2.14, 2.13, 2.12, 2.06, 2.03, 2.02 (s, 7x3H, acetates), 1.88 (s, 3H, *CH*3CONH); FTIR (neat) 2959.2 (C-H), 1816.5, 1745.0 (C=O), 1683.6, 1662.4 (glycal C=C), 1370.6, 1226.9, 1038.7; HRMS (EI) calc. for C39H51NO25K (M+K) 972.2386, found 972.2407.

EXAMPLE 4

α/β Mixture of azidonitrates 7. Compound **6** (370 mg, 0.396 mmol) was dissolved in 2.2 mL of dry acetonitrile and the solution was cooled to -20°C. Sodium azide (NaN_3, 38.6 mg, 0.594, 1.5 eq.) and cerium ammonium nitrate (CAN, 651.3, 1.188 mmol, 3eq.) were added and the mixture was vigorously stirred at -15°C for 12 hrs. The heterogeneous mixture was diluted with ethyl acetate, washed twice with ice cold water and dried over sodium sulphate to provide 400 mg of the crude product. Purification by flash chromatography provided mixture **7** (246 mg, 60 % yield): ^{1}H NMR (400MHz, $CDCl_3$) 6.35 (d, J = 4.2 Hz, 1H, H-1, α-nitrate), 3.79 (s, 3H, methyl ester), 3.41 (dd, J = 4.7, 11.0, 1H, H-2), 2.54 (dd, J = 4.6, 12.8, H-2eq NeuNAc); FTIR (neat) 2117.4 (N3), 1733.9 (C=O); MS (EI) calc. 1037.8, found 1038.4 (M+H).

EXAMPLE 5

α-Azidobromide 8. A solution of the compound **7** (150 mg, 0.145 mmol) in 0.6 mL of dry acetonitrile was mixed with lithium bromide (62.7 mg, 0.725 mmol, 5eq.) and stirred at rt

for 3hrs in the dark. The heterogeneous mixture was diluted with dichloromethane and the solution was washed twice with water, dried over magnesium sulphate and the solvent was evaporated without heating. After flash chromatography (5% MeOH, CH_2Cl_2) α-bromide **8** (120 mg, 75% yield) was isolated and stored under an argon atmosphere at -80°C: ^{1}H NMR (500MHz, $CDCl_3$) δ 6.54 (d, J = 3.7 Hz, 1H, H-1), 3.40 (dd, J = 4.5, 10.8 Hz, 1H, H-2), 2.57 (dd, J = 4.5, 12.9, 1H, H-2eq NeuNAc), 2.20, 2.15, 2.14, 2.12, 2.04, 2.02 (singlets, each 3H, acetates), 1.87 (s, 3H, C*H*3CONH); MS (EI) calc. for C39H51N4BrO25 1055.7, found 1057.4 (M+H).

EXAMPLE 6

Azido-trichloroacetamidate 9. Compound 7 (600mg, 0.578 mmol) was dissolved in 3.6 mL of acetonitrile and the resulting solution was treated with thiophenol (180 μL) and diisopropylethylamine (100μL). After 10 minutes the solvent was removed with a stream of nitrogen. The crude material was purified by chromatography (2-2.5-3-3.5% MeOH/CH_2Cl_2) to provide 472 mg (82%) of intermediate hemiacetal. 60 mg (0.06mmol) of this intermediate was taken up in 200 mL of CH_2Cl_2 and treated with trichloroacetonitrile (60 μL) and 60 mg potassium carbonate. After 6 hrs the mixture is diluted with CH_2Cl_2, solution is removed with a pipette and the excess K_2CO_3 was washed three times with CH_2Cl_2. After evaporation of solvent the crude was purified by flash chromatography (5%MeOH/CH_2Cl_2) to provide **9** (53.2 mg, 64% yield for two steps, 1:1 mixture of α/β anomers). The anomers can be separated by flash chromatography using a graded series of solvent systems (85-90-95-100% EtOAc/hexane).

EXAMPLE 7

Preparation of glycosyl-L-threonine 13 by $AgClO_4$-promoted glycosidation with glycosyl bromide 8. A flame dried flask is charged with silver perchlorate (27.3 mg, 2 eq), 115 mg of 4Å molecular sieves and N-FMOC-L-threonine benzyl ester (37.3 mg, 0.086 mmol, 1.2 eq) in the dry box. 0.72 mL of CH_2Cl_2 was added to the flask and the mixture was stirred

at rt for 10 minutes. Donor **8** (76 mg, 0.072 mmol) in 460 μL of CH_2Cl_2 was added slowly over 40 minutes. The reaction was stirred under argon atmosphere at rt for two hours. The mixture was then diluted with CH_2Cl_2 and filtered through celite. The precipitate was thoroughly washed with CH_2Cl_2, the filtrate was evaporated and the crude material was purified on a silica gel column (1-1.5-2-2.5% MeOH/CH_2Cl_2) to provide **13** (74mg, 74% yield). The undesired β-anomer was not detected by ^{1}H NMR and HPLC analysis of the crude material. **13**: ^{1}H NMR (500MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 7.2 Hz, 2H), 7.40 - 7.25 (m, 8H), 5.72 (d, 9.2 Hz, 1H), 5.46 (s, 1H), 5.33 (m, 1H), 5.29 (d, J = 8.2 Hz, 1H), 5.23 (s, 2H), 5.11 - 5.04 (m, 3H), 4.87 - 4.71 (m, 4H), 4.43 - 4.39 (m, 3H), 4.33 - 4.25 (m, 4H), 4.09 - 3.97 (m, 6H), 3.79 (s, 3H, methyl ester), 3.66 (dd, J = 3.7, 10.6 Hz, 1H, H-3), 3.38 (dd, J = 3.0, 10.7 Hz, 1H, H-2), 2.52 (dd, J = 4.3, 12.7, 1H, H-2eq NeuNAc), 2.20, 2.13, 2.11, 2.10, 2.04, 2.03, 2.02 (singlets, 3H, acetates), 1.87 (s, 3H, *CH*3CONH), 1.35 (d, J = 6.15 Hz, Thr-CH_3); FTIR (neat) 2110.3 (N3), 1748.7 (C=O), 1223.9, 1043.6; HRMS (EI) calc. for C65H75N5O30K (M+K) 1444.4130, found 1444.4155.

EXAMPLE 8

Glycosyl-L-serine 12.

BF_3·OEt_2 promoted glycosydation with trichloroacetamidate 9: A flame dried flask is charged with donor **9** (50 mg, 0.044 mmol), 80 mg of 4Å molecular sieves and N-FMOC-L-serine benzyl ester (27.5 mg, 0.066 mmol) in the dry box. 0.6 mL of THF was added to the flask and the mixture was cooled to -30°C. BF_3·OEt_2 (2.8 mL, 0.022 mmol, 0.5 eq.) was added and the reaction was stirred under argon atmosphere. During three hours the mixture was warmed to -10°C and then diluted with EtOAc and washed with sat sodium bicarbonate while still cold. The crude material was purified on silica gel column (2-2.5-3% MeOH/CH_2Cl_2) to provide **12** (40 mg, 66% yield) as a 4:1 mixture of α:β isomers. The pure α-anomer was separated by flash chromatography (80-85-90-100% EtOAc/ hexane).

EXAMPLE 9

Glycosyl-L-threonine (15). Compound **13** (47 mg, 33.42 μmol) was treated with thiolacetic acid (3 mL, distilled three times) for 27 hrs at rt. Thiolacetic acid was removed with a stream of nitrogen, followed by toluene evaporation (four times). The crude product was purified by flash chromatography (1.5-2-2.5-3-3.5% MeOH/CH_2Cl_2) to yield 37 mg (78%) of an intermediated which was immediately dissolved in 7.6 mL of methanol and 0.5 mL of water. After purging the system with argon 6.5 mg of palladium catalyst (10% Pd-C) was added and hydrogen balloon was attached. After 8 hrs hydrogen was removed by argon atmosphere, the catalyst was removed by filtration through filter paper and the crude material was obtained upon removal of solvent. Flash Chromatography (10% MeOH/CH_2Cl_2) provided pure compound **15** (36 mg, 78%): ^{1}H NMR (500MHz, $CDCl_3$) mixture of rotamers, characteristic peaks δ 3.80 (s, 3H, methyl ester), 3.41 (m, 1H, H-2), 2.53 (m, 1H, H-2e NeuNAc)), 1.45 (d, J = 5.1 Hz, Thr-CH_3), 1.35 (d, J = 5.8 Hz, Thr-CH3); FTIR (neat) 1818.2, 1747.2 (C=O), 1371.1, 1225.6, 1045.0; HRMS (EI) calc. for C60H73N3O31K (M+K) 1370.3870, found 1370.3911.

EXAMPLE 10

Glycosyl-L-serine (14). The compound **14** was prepared in 80% yield from **12** following the same procedure as for **15**.

EXAMPLE 11

General procedure for peptide coupling:

Glycosyl amino acid **14** or **15** (1eq) and the peptide with a free amino group (1.2 eq) were dissolved in CH_2Cl_2 (22 mL/1 mmol). The solution was cooled to 0°C and IIDQ (1.15 -1.3 eq.) is added (1mg in ca 20mL CH_2Cl_2). The reaction was then stirred at rt for 8 hrs. The mixture was directly added to the silica gel column.

EXAMPLE 12

General procedure for FMOC deprotection:

A substrate (1mmol in 36 mL DMF) was dissolved in anhydrous DMF followed by addition of KF (10eq) and 18-crown-6 ether (catalytic amount). The mixture was then stirred for 48 hrs at rt. Evaporation of DMF *in vacuo* was followed by flash chromatography on silica gel.

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EXAMPLE 13

Glycopeptide 16. ^{1}H NMR (500MHz, $CDCl_3$) δ 3.45 - 3.30 (m, 3x1H, H-2), 3.74 (s, 3H, methyl ester), 2.58 - 2.49 (m, 3x1H, H-2eq NeuNAc); FTIR (neat) 2961.7, 1819.2, 1746.5, 1663.5, 1370.5, 1225.7, 1042.5; MS (EI) calc. 3760, found 1903.8 / doubly charged = 3806 (M+2Na).

10

EXAMPLE 14

Glycopeptide 1. ^{1}HNMR (500 MHz, D_2O) δ 4.73 (m, 2H, 2xH-1), 4.70 (d, 1H, H-1), 4.64 (m, 3H, 3xH-1'), 4.26 -4.20 (m, 5H), 4.12 - 4.00 (m, 7H), 3.95 - 3.82 (7H), 3.77 - 3.27 (m, 51H), 2.55 - 2.51 (m, 3H, 3xH-2eq NeuNAc), 1.84 - 1.82 (m, 21H, CH3CONH), 1.52 - 1.45 (m, 3H, H-2ax NeuNAc), 1.20 (d, J = 7.2 Hz, 3H), 1.18 (d, J = 6.6 Hz, 3H), 1.12 (d, J = 6.2 Hz, 3H), 0.71 (d, J = 6.6 Hz, 6H, val); 13C NMR (500MHz, D2O) anomeric carbons: 105.06, 105.01, 100.60, 100.57, 100.53, 100.11, 99.52, 98.70; MS (FAB) C96H157N11O64 2489 (M+H); MS(MALDI) 2497.

15

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EXAMPLE 15

Glycopeptide 19. MS (EI) calc. for C178H249N15O94Na2 4146 (M+2Na), found 4147, negative ionization mode confirmed the correct mass; MALDI (Matrix Assisted Laser Desorption Ionization) provided masses 4131, 4163.

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EXAMPLE 16

Glycopeptide 20:

MS (FAB) C119H193N15O70N 2975 (M+Na)

EXAMPLE 17

Preparation of azid nitrates 4': To a solution of protected galactal **3'** (4.14 g, 12.1 mmol) in 60 ml of anhydrous CH_3CN at -20 °C was added a mixture of NaN_3 (1.18 g, 18.1 mmol) and CAN (19.8 g, 36.2 mmol). The reaction mixture was vigorously stirred at -20 °C for overnight. Then the reaction mixture was diluted with diethyl ether, and washed with cold water and brine subsequently. Finally, the solution was dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel. A mixture of α- and β-isomers (**4'**) (2.17 g, 40% yield) was obtained. The ratio of α-isomer and β-isomer was almost 1:1 based on ^1H NMR. **4a'**: $[\alpha]_D^{20}$ 94.5°(c 1.14, $CHCl_3$); FT-IR (film) 2940, 2862, 2106, 1661, 1460, 1381, 1278 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.34 (d, J=3.9Hz, 1H), 4.34(m, 2H), 4.21 (t, J=6.4Hz, 1H), 3.95 (dd, J=9.6, 7.2Hz, 1H), 3.85 (dd, J=9.6, 6.4Hz, 1H), 3.78 (m, 1H), 1.52 (s, 3H), 1.35 (s, 3H), 1.04 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.29, 97.02, 73.36, 71.89, 71.23, 61.95, 59.57, 28.18, 25.96, 17.86, 11.91; HRMS(FAB) calc. for $C_{18}H_{34}N_4O_7SiK$ [M+K$^+$] 485.1833, found 485.1821. **4b'**: $[\alpha]_D^{20}$ 27.9° (c 1.28, $CHCl_3$); FT-IR (film) 2940, 2862, 2106, 1666, 1459, 1376, 1283 cm^{-1}; ^1H NMR (300 MHz, CDC_{l3}) δ 5.50 (d, J=8.9Hz, 1H), 4.30 (dd, J=4.3, 1.5Hz, 1H), 4.15 (dd, J=6.2, 4.3Hz, 1H), 3.89-4:03 (m, 3H), 3.56 (dd, J=8.9, 7.3Hz, 1H), 1.58 (s, 3H), 1.38 (s, 3H), 1.08 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.90, 98.09, 77.53, 74.58, 71.99, 61.82, 61.68, 28.06, 25.97, 17.85, 11.89; HRMS (FAB) calc. for $C_{18}H_{34}N_4O_7SiK$ [M+K$^+$] 485.1833, found 485.1857.

EXAMPLE 18

Preparation of trichloroacetimidates 5a' and 5b': To a solution of a mixture of azidonitrates (**4'**) (1.36 g, 3.04 mmol) in 10 ml of anhydrous CH_3CN at 0 °C were slowly added $Et(i\text{-}Pr)_2N$ (0.53 ml, 3.05 mmol) and PhSH (0.94 ml, 9.13 mmol) subsequently. The reaction mixture was stirred at 0 °C for 1 hour, then the solvent was evaporated at room temperature in vacuo. The residue was separated by chromatography on silica gel to give the hemiacetal (1.22 g, 99.8% yield). To a solution of this hemiacetal (603 mg, 1.50 mmol)

in 15 ml of anhydrous CH_2Cl_2 at 0°C were added K_2CO_3 (1.04 g, 7.50 mmol) and CCl_3CN (1.50 ml, 15.02 mmol). The reaction mixture was stirred from 0°C to room temperature for 5 hours. The suspension was filtered through a pad of celite and washed with CH_2Cl_2. The filtrate was evaporated and the residue was separated by chromatography on silica gel to give α-trichloroacetimidate **5a'** (118 mg, 14% yield), β-trichloroacetimidate **5b'** (572 mg, 70% yield) and recovered hemiacetal (72 mg). **5a'**: $[\alpha]_D^{20}$ 84.0° (c 1.02, $CHCl_3$); FT-IR (film) 2942, 2867, 2111, 1675, 1461, 1381, 1244 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 6.29 (d, J=3.3Hz, 1H), 4.47 (dd, J=8.0, 5.3Hz, 1H), 4.39 (dd, J=5.3, 2.4Hz, 1H), 4.25 (m, 1H), 3.97 (dd, J=9.5, 7.8Hz, 1H), 3.87 (dd, J=9.5, 6.0Hz, 1H), 3.67 (dd, J=8.0, 3.3Hz, 1H), 1.53 (s, 3H), 1.36 (s, 3H), 1.04 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.67, 109.98, 94.72, 77.20, 73.35, 72.11, 70.83, 62.01, 60.80, 28.29, 26.09, 17.88, 11.88; HRMS (FAB) calc. for $C_{20}H_{35}N_4O_5SiKCl_3$ [M+K⁺] 583.1080, found 583.1071.

5b': $[\alpha]_D^{20}$ 30.6° (c 1.12, $CHCl_3$); FT-IR (film) 2941, 2110, 1677, 1219 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1H), 5.57 (d, J=9.0Hz, 1H), 4.27 (d, J=5.2Hz, 1H), 3.95-4.02 (m, 4H), 3.63 (t, J=9.0Hz, 1H). 1.57 (s, 3H), 1.34 (s, 3H), 1.04 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.94, 110.55, 96.47, 77.20, 74.58, 72.21, 64.84, 61.89, 28.29, 26.07, 17.87, 11.90; HRMS (FAB) calc. for $C_{20}H_{35}N_4O_5SiKCl_3$ [M+K⁺] 583.1080, found 583.1073.

EXAMPLE 19

Preparation of glycosyl fluorides 6a' and 6b': To a solution of the hemiacetal prepared previously (68.0 mg, 0.169 mmol) in 3 ml of anhydrous CH_2Cl_2 at 0 °C was added DAST (134 ml, 1.02 mmol) slowly. The reaction mixture was stirred at 0 °C for 1 hour. Then the mixture was diluted with EtOAc, washed with sat. $NaHCO_3$ and brine subsequently. Finally, the solution was dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give α-fluoride **6a'** (30.2 mg, 44% yield) and β-fluoride **6b'** (33.7 mg, 49% yield). **6a'**: $[\alpha]_D^{20}$ 689.5° (c 1.47, $CHCl_3$); FT-IR (film) 2944, 2867, 2115, 1462, 1381 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.59 (dd, J=53.0, 2.6Hz, 1H), 4.34-4.40 (m, 2H), 4.26 (m, 1H), 3.96 (t, J=9.3Hz, 1H), 3.88 (dd,

J=9.3, 6.0Hz, 1H), 3.48 (ddd, J=25.5, 7.0, 2.6Hz, 1H), 1.50 (s, 3H), 1.34 (s, 3H), 1.05 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.03, 107.45, 104.46, 77.21, 76.38, 73.21, 71.79, 70.48, 61.88, 61.23, 60.91, 28.17, 26.03, 17.09, 11.92; HRMS (FAB) calc. for $C_{18}H_{35}N_3O_4SiF$ [M+H$^+$] 404.2378, found 404.2369.

6b': $[\alpha]_D^{20}$ 153.8^0 (c 1.65, $CHCl_3$); FT-IR (film) 2943, 2867, 2116, 1456, 1382, 1246 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.05 (dd, J=52.6, 7.4Hz, 1H), 4.27 (dt, J=5.5, 2.0Hz, 1H), 3.89-4.05 (m, 4H), 3.70 (dt, J=12.3, 5.1Hz, 1H), 1.53 (s, 3H), 1.32 (s, 3H), 1.04 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.64, 109.09, 106.24, 76.27, 76.16, 73.42, 71.63, 64.80, 64.52, 61.77, 27.80, 25.78, 17.03, 11.86; HRMS (FAB) calc. for $C_{18}H_{35}N_3O_4SiF$ [M+H$^+$] 404.2378, found 404.2373.

EXAMPLE 20

Coupling of β-trichloroacetimidate 5b' with protected serine derivative 7': Synthesis of 9a' and 9b': To a suspension of β-trichloroacetimidate **5b'** (52.3 mg, 0.096 mmol), serine derivative 7' (44.0 mg, 0.105 mmol) and 200 mg 4Å molecular sieve in a mixture of 2 ml of anhydrous CH_2Cl_2 and 2 ml of anhydrous hexane at -78 °C was added a solution of TMSOTf (1.91 μl, 0.01 mmol) in 36 μl of CH_2Cl_2. The reaction mixture was stirred at -78 °C for a half hour, then warmed up to room temperature for 3 hours. The reaction was quenched by Et_3N. The suspension was filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give_α-product **9a'** (55 mg, 71% yield) and β-product **9b'** (22 mg, 29% yield). **9a'**: $[\alpha]_D^{20}$ 70.5^0 (c 2.0, $CHCl_3$); FT-IR (film) 3433, 3348, 2943, 2867, 2109, 1730 , 1504, 1453, 1381, 1336 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J=7.5Hz, 2H), 7.57 (d, J=7.5Hz, 2H), 7.25-7.40 (m, 9H), 5.73 (d, J=8.4Hz, 1H), 5.24 (d, J=12.1Hz, 1H), 5.17 (d, J=12.1, 1H), 4.73 (d, J=3.2Hz, 1H), 4.60 (m, 1H), 4.41 (dd, J=10.2, 7.2Hz, 1H), 4.20-4.31 (m, 4H), 3.82-3.98 (m, 5H), 3.23 (dd, J=8.0, 3.2Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H), 1.02 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.65, 155.88, 143.81, 143.73, 141.27, 135.04, 128.63,

128.54, 127.71, 127.60, 125.18, 125.11, 109.67, 98.71, 77.23, 72.88, 72.39, 68.95, 68.79, 67.73, 67.36, 62.28, 61.10, 54.39, 47.08, 28.26, 26.10, 17.91, 11.90; HRMS (FAB) calc. for $C_{43}H_{56}N_4O_9SiK$ [M+K$^+$] 839.3453, found 839.3466, 839.3453 ;

9b': $[\alpha]_D^{20}$ 20.6° (c 1.05, $CHCl_3$); FT-IR (film) 3433, 2943, 2866, 2114, 1729, 1515, 1453, 1382 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, J=7.4Hz, 2H), 7.63 (t, J=7.4Hz, 2H), 7.30-7.44 (m, 9H), 5.91 (d, J=8.4Hz, 1H), 5.30 (d, J=12.4Hz, 1H), 5.26 (d, J=12.4Hz, 1H), 4.65 (m, 1H), 4.48 (dd, J=10.0, 2.6Hz, 1H), 4.39 (t, J=7.4Hz, 2H), 4.23-4.28 (m, 3H), 3.89-4.04 (m, 3H), 3.85 (dd, J=10.0, 3.1Hz, 1H), 3.78 (m, 1H), 3.41 (t, J=8.2Hz, 1H), 1.58 (s, 3H), 1.36 (s, 3H), 1.08 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.37, 155.92, 143.90, 143.69, 141.25, 135.27, 128.55, 128.27, 127.94, 127.68, 127.07, 125.27, 125.21, 119.94, 110.37, 102.30, 76.87, 73.78, 72.19, 69.68, 67.40, 67.33, 65.44, 61.99, 54.20, 47.06, 28.32, 26.10, 17.89, 11.88; HRMS (FAB) calc. for $C_{43}H_{56}N_4O_9SiK$ [M+K$^+$] 839.3453, found 839.3466.

EXAMPLE 21

Coupling of β-trichloroacetimidate 5b' with protected serine derivative 7' in THF Promoted by TMSOTf (0.5 eq.): To a suspension of trichloroacetimidate **5b'** (14.4 mg, 0.027 mmol), serine derivative **7'** (16.7 mg, 0.040 mmol) and 50 mg 4Å molecular sieve in 0.2 ml of anhydrous THF at -78 °C was added a solution of TMSOTf (2.7 μl, 0.013 mmol) in 50 μl of THF. The reaction was stirred at -78 °C for 2 hours and neutralized with Et_3N. The reaction mixture was filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give the α-product **9a'** (18.5 mg, 86% yield).

EXAMPLE 22

Coupling of α-trichloroacetimidate 5a with protected serine derivative 7' in THF Promoted by TMSOTf (0.5eq.): To a suspension of trichloroacetimidate **5a'** (12.3 mg,

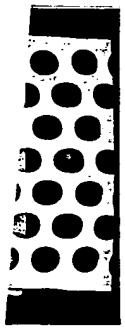
0.023 mmol), serine derivative 7' (14.1 mg, 0.034 mmol) and 50 mg 4Å molecular sieve in 0.2 ml of anhydrous THF at -78 °C was added a solution of TMSOTf (2.2 μl, 0.011 mmol) in 45 μl of THF. The reaction was stirred at -78 °C for 4 hours and neutralized with Et_3N. The reaction mixture was filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give the α-product **9a'** (11.8 mg, 66% yield).

EXAMPLE 23

Coupling of β-trichloroacetimidate 5b' with protected threonine derivative 8: Synthesis of 10a' and 10b': To a suspension of β-trichloroacetimidate **5b'** (50.6 mg, 0.093 mmol), threonine derivative **8'** (44.0 mg, 0.102 mmol) and 200 mg 4Å molecular sieve in a mixture of 2 ml of anhydrous CH_2Cl_2 and 2 ml of anhydrous hexane at -78 °C was added a solution of TMSOTf (1.85 μl, 0.009 mmol) in 35 μl of CH_2Cl_2. The reaction mixture was stirred at -78 °C for a half hour, then warmed up to room temperature for 4 hours. The reaction was quenched by Et_3N. The suspension was filtered through a pad of celite and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give recovered threonine derivative **7'** (28.0 mg), the α-product **10a'** (22.0 mg, 29% yield) and the β-product **10b'** (3.0 mg, 4% yield). **10a'**: $[\alpha]_D^{20}$ 55.2° (c 0.88, $CHCl_3$); FT-IR (film) 3430, 2941, 2866, 2109, 1730, 1510, 1452, 1380 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J=7.5Hz, 2H), 7.59 (d, J=7.5Hz, 2H), 7.26-7.41 (m, 9H), 5.62 (d, J=9.4Hz, 1H), 5.22 (d, J=12.3Hz, 1H), 5.18 (d, J=12.3Hz, 1H), 4.73 (d, J=3.6Hz, 1H), 4.36-4.47 (m, 3H), 4.19-4.32 (m, 4H), 4.09 (m, 1H), 3.91 (dd, J=9.8, 6.6Hz, 1H), 3.83 (dd, J=9.8, 5.5Hz, 1H), 3.24 (dd, J=8.1, 3.6Hz, 1H), 1.49 (s, 3H), 1.33 (s, 3H), 1.32 (d, J=6.0Hz, 3H), 1.05 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.12, 156.74, 143.94, 143.69, 141.29, 135.00, 128.65, 128.59, 127.70, 127.10, 125.19, 119.96, 109.78, 99.09, 77.22, 73.16, 72.53, 69.03, 67.71, 67.40, 62.54, 61.61, 58.84, 47.15, 28.32, 26.17, 18.76,

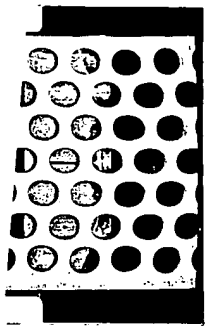
17.94, 11.92; HRMS (FAB) calc. for $C_{44}H_{58}N_4O_9SiK$ [M+K$^+$] 853.3608, found 853.3588;

10b': $[\alpha]_D^{20}$ 92.4° (c 0.47, CH_2Cl_2); FT-IR (film) 3434, 3351, 2940, 2865, 2111, 1728, 1515, 1455 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J=7.5Hz, 2H), 7.59 (t, J=7.5Hz, 2H). 7.25-7.40 (m, 9H), 5.68 (d, J=9.3Hz, 1H), 5.20 (d, J=12.4Hz, 1H), 5.17 (d, J=12.4Hz, 1H), 4.58 (m, 1H), 4.47 (dd, J=9.3, 3.4Hz, 1H), 4.34 (d, J=7.8Hz, 2H), 4.18-4.29 (m, 3H), 3.96 (t, J=8.9Hz, 1H), 3.84 (dd, J=10.0, 5.2Hz, 1H), 3.81 (dd, J=8.2, 5.2Hz, 1H), 3.65 (m, 1H), 3.34 (t, J=8.1Hz, 1H), 1.55 (s, 3H), 1.32 (s, 3H), 1.30 (d, J=6.4Hz, 3H), 1.02 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.89, 156.73, 143.96, 143.73, 141.27, 135.38, 128.61, 128.27, 127.93, 127.67, 127.08, 125.26, 119.93, 110.26, 99.32, 77.91, 77.82, 74.03, 73.55, 72.01, 67.42, 67.25, 65.32, 61.66, 58.61, 47.12, 28.36, 26.08, 17.88, 16.52, 11.87; HRMS(FAB) calc. for $C_{44}H_{58}N_4O_9SiNa$ [M+Na$^+$] 837.3869, found 837.3887.



EXAMPLE 24

Coupling of α-glycosyl fluoride 6a' with protected threonine derivative 8' in CH_2Cl_2 promoted by $(Cp)_2ZrCl_2$-$AgClO_4$: To a suspension of $AgClO_4$ (25.1 mg, 0.121 mmol), $Cp)_2ZrCl_2$ (17.8 mg, 0.06 mmol) and 150 mg 4Å molecular sieve in 1 ml of anhydrous $:H_2Cl_2$ at -30 °C was added a solution of α-glycosyl fluoride **6a'** (16.3 mg, 0.04 mmol) and reonine derivative **8'** (19.2 mg, 0.045 mmol) in 4.0 ml of anhydrous CH_2Cl_2 slowly. The ıction was stirred at -30 °C for 6 hours and quenched with sat. $NaHCO_3$. The solution s filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, esidue was separated by chromatography on silica gel to give the α-product **10a'** mg, 75% yield) and the β-product **10b'** (3.9 mg, 12% yield).



EXAMPLE 25

ing of β-glycosyl fluoride 6b' with protected threonine derivative 8' in CH_2Cl_2 ted by $(Cp)_2ZrCl_2$-$AgClO_4$: To a suspension of $AgClO_4$ (24.4 mg, 0.118 mmol), Cl_2 (17.2 mg, 0.059 mmol) and 200 mg 4Å molecular sieve in 1 ml of anhydrous

CH_2Cl_2 at -30 °C was added a solution of β-glycosyl fluoride **6b'** (15.8 mg, 0.03918 mmol) and threonine derivative **8'** (20.3 mg, 0.04702 mmol) in 4.0 ml of anhydrous CH_2Cl_2 slowly. The reaction was stirred at -30 °C for 10 hours and quenched with sat. $NaHCO_3$. The solution was filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with sat. $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give the α-product **10a'** (22.3 mg, 70% yield) and the β-product **10b'** (3.9 mg, 12% yield).

EXAMPLE 26

Deprotection of the silyl group of 9a': To a solution of the α-product **9a'** (15.0 mg, 0.01873 mmol) in 2 ml of THF at 0 °C were added HOAc (56 μl, 0.978 mmol) and 1M TBAF (240 μl, 0.240 mmol). The reaction was run at 0 °C for 1 hour, and then warmed up to room temperature for 3 days. The mixture was diluted with EtOAc, washed with H_2O, brine, and finally dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give desired product **11'** (12.4 mg, 100%). **11'**: $[\alpha]_D^{20}$ 78.3° (c 0.67, CH_2Cl_2); FT-IR (film) 3432, 3349, 2987, 2938, 2109, 1729, 1517, 1452, 1382 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J=7.5Hz, 2H), 7.59 (d, J=7.5Hz, 2H), 7.27-7.41 (m, 9H), 6.01 (d, J=9.2Hz, 1H), 5.21 (d, J=12.4Hz, 1H), 5.18 (d, J=12.4Hz, 1H), 4.74 (d, J=3.3Hz, 1H), 4.58 (m, 1H), 4.41 (d, J=7.0Hz, 2H), 4.14-4.23 (m, 3H), 4.02 (dd, J=5.4, 2.4Hz, 1H), 3.91-3.97 (m, 2H), 3.68-3.85 (m, 2H), 3.27 (dd, J=8.2, 3.3Hz, 1H), 1.48 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.71, 155.85, 143.78, 143.71, 141.32, 135.03, 128.59, 127.72, 127.08, 125.08, 119.99, 110.20, 99.12, 77.20, 73.35, 73.11, 70.22, 68.54, 67.76, 67.04, 62.48, 60.73, 54.66, 47.12, 28.10, 26.14; HRMS (FAB) calc. for $C_{34}H_{37}N_4O_9$ [M+H$^+$] 645.2560, found 645.2549.

EXAMPLE 27

Deprotection of the silyl group of 10a': To a solution of the α-product **10a'** (16.0 mg, 0.02 mmol) in 3 ml of THF at 0 °C were added HOAc (67 μl, 1.18 mmol) and 1M TBAF (300 μl,

0.3000 mmol). The reaction was run at 0 °C for 1 hour, and then warmed up to room temperature for 3 days. The mixture was diluted with EtOAc, washed with H_2O, brine, and finally dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give desired product **12'** (12.1 mg, 94%). **12'**: $[\alpha]_D^{20}$ 731.8° (c 0.62, CH_2Cl_2); FT-IR (film) 3430, 2986, 2936, 2109, 1728, 1515, 1451, 1382 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J=7.4Hz, 2H), 7.60 (d, J=7.4Hz, 2H), 7.25-7.41 (m, 9H), 5.67 (d, J=9.0Hz, 1H), 5.21 (br.s, 2H), 4.82 (d, J=3.2Hz, 1H), 4.40-4.52 (m, 3H), 4.33-4.38 (m, 2H), 4.19-4.29 (m, 2H), 4.09 (m, 1H), 3.75-3.92 (m, 2H), 3.30 (dd, J=8.0, 3.2Hz, 1H), 2.04 (m, 1H), 1.50 (s, 3H), 1.35 (s, 3H), 1.30 (d, J=6.2Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.13, 156.69, 143.91, 143.69, 141.30, 134.98, 128.61, 127.72, 127.10, 125.20, 119.97, 110.25, 98.39, 76.26, 73.49, 68.35, 67.75, 67.36, 62.62, 61.31, 58.69, 47.16, 28.18, 26.24, 18.54; HRMS (FAB) calc. for $C_{35}H_{39}N_4O_9$ [M+H$^+$] 659.2716, found 659.2727.

EXAMPLE 28

Preparation of compound 14': To a suspension of trichloroacetimidate **13'** (332.0 mg, 0.435 mmol), the acceptor **11'** (140.2 mg, 0.218 mmol) and 1.0 g 4Å molecular sieve in 4 ml of anhydrous CH_2Cl_2 at -30 °C was added a solution of $BF_3{\cdot}Et_2O$ (13.8 μl, 0.109 mmol) in 120 μl of anhydrous CH_2Cl_2 slowly. The reaction mixture was stirred at -30 °C for overnight, then warmed up to room temperature for 3 hours. The reaction was quenched with Et_3N, filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give crude recovered acceptor **11'** which was further converted to compound **9a'** (87.0 mg, 0.109 mmol) and crude coupling product which was further reduced to compound **14'** by pyridine and thiolacetic acid. The crude coupling product was dissolved in 1 ml of anhydrous pyridine and 1 ml of thiolacetic acid at 0 °C. The reaction mixture was stirred at room temperature for overnight. The solvent was evaporated in vacuo at room temperature and

the residue was separated by chromatography on silica gel to give compound **14'** (99.6 mg, 72% yield based on 50% conversion of acceptor **11'**). **14'**: $[\alpha]_D^{20}$ 267.9° (c 4.0 , $CHCl_3$); FT-IR (film) 3361, 3018, 1751, 1672, 1543, 1452, 1372 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J=7.5Hz, 2H), 7.58 (m, 2H), 7.26-7.38 (m, 9H), 6.26 (d, J=8.2Hz, 1H), 5.83 (d, J=9.3Hz, 1H), 5.59 (d, J=9.2Hz, 1H), 5.32 (d, J=2.7Hz, 1H), 5.16 (s, 2H), 5.02-5.11 (m, 2H), 4.94 (dd, J=10.4, 3.4Hz, 1H), 4.59 (d, J=3.4Hz, 1H), 4.35-4.52 (m, 6H), 3.60-4.19 (m, 16H), 2.11 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.93 (s, 3H), 1.91 (s, 3H), 1.83 (s, 3H), 1.48(s, 3H), 1.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.33, 170.23, 170.15, 170.07, 169.94, 169.85, 169.19, 155.92, 143.75, 143.64, 141.22, 135.12, 128.62, 128.39, 127.67, 127.01, 124.99, 119.93, 109.81, 101.12, 100.84, 98.14, 77.21, 75.49, 74.28, 72.61, 72.12, 70.74, 69.10, 68.80, 67.61, 67.38, 67.28, 67.09, 66.64, 62.28. 60.77, 54.25, 53.03, 50.09, 47.09, 27.76, 26.40, 23.18, 23.03, 20.71, 20.47, 20.36; HRMS (FAB) calc. for $C_{62}H_{75}N_3O_{26}Na$ [M+Na$^+$] 1300.4539, found 1300.4520 .

EXAMPLE 29

Preparation of compound 15': To a suspension of trichloroacetimidate **13'** (305.0 mg, 0.3996 mmol), the acceptor **12'** (131.6 mg, 0.1998 mmol) and 1.0 g 4Å molecular sieve in 4 ml of anhydrous CH_2Cl_2 at -30 °C was added a solution of BF_3Et_2O (12.7 μl, 0.10 mmol) in 115 μl of anhydrous CH_2Cl_2 slowly. The reaction mixture was stirred at -30 °C for overnight, then warmed up to room temperature for 3 hours. The reaction was quenched with Et_3N, filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give crude recovered acceptor **12'** which was further converted to compound **10a'** (85.0 mg, 0.104 mmol) and crude coupling product which was further reduced to compound **15'** by pyridine and thiolacetic acid. The crude coupling product was dissolved in 1 ml of anhydrous pyridine and 1 ml of thiolacetic acid at 0 °C. The reaction mixture was stirred at room temperature for overnight. The solvent was evaporated in vacuo at room temperature and

the residue was separated by chromatography on silica gel to give compound **15'** (71.1 mg, 58% yield based on 48% conversion of acceptor **12'**). **15'**: $[\alpha]_D^{20}$ 346.8° (c 0.53, $CHCl_3$); FT-IR (film) 3366, 2986, 1750, 1673, 1541, 1452, 1372 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J=7.4Hz, 1H), 7.57 (d, J=7.4Hz, 2H), 7.27-7.45 (m, 9H), 5.83 (d, J=9.4Hz, 1H), 5.74 (d, J=9.4Hz, 1H), 5.61 (d, J=8.9Hz, 1H), 5.31 (d, J=3.0Hz, 1H), 4.91-5.16 (m, 5H), 4.62 (d, J=3.2Hz, 1H), 4.32-4.46 (m, 6H), 3.95-4.22 (m, 11H), 3.64-3.84 (m, 3H), 3.57 (m, 1H), 2.12 (s, 6H), 2.10 (s, 3H), 2.06 (s, 3H), 2.01 (s, 6H), 1.93 (s, 3H), 1.86 (s, 3H), 1.51 (s, 3H), 1.26 (s, 3H), 1.22 (d, J=5.5Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.70, 170.38, 170.19, 169.94, 169.86, 169.74, 169.20, 156.34, 143.72, 143.59, 141.26, 134.59, 128.74, 128.37, 127.71, 127.03, 124.92, 119.94, 109.76, 101.48, 100.86, 99.48, 77.20, 76.23, 75.49, 74.41, 72.74, 72.43, 70.76, 69.26, 69.13, 67.56, 67.45, 67.13, 66.65, 62.29, 60.78, 58.47, 52.83, 50.35, 47.16, 27.86, 26.54, 23.22, 23.03, 20.72, 20.49, 20.37, 18.20; HRMS (FAB) calc. for $C_{63}H_{78}N_3O_{26}$ $[M+H^+]$ 1292.4871, found 1292.4890.

EXAMPLE 30

Synthesis of compound 1': The trisaccharide **14'** (105.8 mg, 0.083 mmol) was dissolved in 5 ml of 80% aq. HOAc at room temperature. The reaction mixture was stirred at room temperature for overnight, then at 40 °C for 3 hours. The solution was extracted with EtOAc, washed with sat. $NaHCO_3$, H_2O, brine, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give diol (93.0 mg, 91% yield). To a solution of this diol (91.5 mg, 0.074 mmol) in 10 ml of anhydrous CH_2Cl_2 at 0 °C were added catalytic DMAP (4.5 mg, 0.037 mmol), Et_3N (103 μl, 0.74 mmol) and Ac_2O (28 μl, 0.30 mmol) subsequently. The reaction was run for overnight at room temperature. The reaction mixture was diluted with EtOAc, washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give peracetylated compound (88.8 mg, 91%yield). To a suspension of 10% Pd/C (5.0 mg) in a mixture of 1 ml of MeOH and 0.1 ml of H_2O was added a solution of the peracetylated compound (38.5 mg, 0.03 mmol) in

4.0 ml of MeOH. The reaction was stirred under H_2 atmosphere at room temperature for 4 hours. The reaction mixture was passed through a short column of silica gel to remove the catalyst and washed with MeOH. After removal of the solvent, the residue was dissolved in 1.5 ml of DMF and to this solution was added 0.5 ml of morpholine at 0 °C slowly. The reaction was stirred at room temperature for overnight. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on silica gel to give 29.0 mg material which was further deacetylated in basic condition. The material got previously was dissolved in 50 ml of anhydrous THF and 5 ml of anhydrous MeOH. The solution was cooled to 0 °C and to this solution was added a solution of NaOMe (14.0 mg, 0.26 mmol) in 5 ml of anhydrous MeOH. The reaction was stirred at room temperature for overnight and quenched with 50% aq. HOAc. After evaporation of the solvent, the residue was separated by chromatography on reverse-phase silica gel to give crude product, which was further purified by gel permeation filtration on Sephadex LH-20 to give the final product **1'** (15.1 mg, 77%yield). **1'**: $[\alpha]_D^{20}$ 715.6° (c 0.1, H_2O); ^1H NMR (300MHz, CD_3OD-D_2O) δ 4.85 (d, J=3.4Hz, 1H), 4.55 (d, J=7.4Hz, 1H), 4.46 (d, J=7.0Hz, 1H), 4.26 (dd, J=10.9, 3.5Hz, 1H), 3.34-4.09 (m, 20H), 2.07 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD-D_2O) δ 175.64, 175.36, 104.61, 102.98, 99.57, 80.35, 76.94, 76.36, 74.32, 73.88, 72.57, 71.30, 70.82, 70.16, 69.21, 62.50, 61.62, 56.64, 51.58, 51.22, 23.63, 23.40; HRMS(FAB) calc. for $C_{25}H_{44}N_3O_{18}$ [M+H$^+$] 674.2620, found 674.2625.

EXAMPLE 31

Synthesis of compound 2': The trisaccharide **15'** (70.2 mg, 0.054 mmol) was dissolved in 5 ml of 80% aq. HOAc at room temperature. The reaction mixture was stirred at room temperature for overnight, then at 40 °C for 3 hours. The solution was extracted with EtOAc, washed with sat. $NaHCO_3$, H_2O, brine, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give diol (67.1 mg, 99% yield). To a solution of diol (65.1 mg, 0.052 mmol) in 8 ml of anhydrous CH_2Cl_2 at 0 °C were added catalytic DMAP (3.2 mg, 0.026 mmol), Et_3N (72 μl,

0.52 mmol) and Ac_2O (20 μl, 0.21 mmol) subsequently. The reaction was run for overnight at room temperature. The reaction mixture was diluted with EtOAc, washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give peracetylated compound (66.0 mg, 95%yield). To a suspension of 10% Pd/C (5.0 mg) in a mixture of 1 ml of MeOH and 0.1 ml of H_2O was added a solution of the peracetylated compound (22.1 mg, 0.017 mmol) in 4.0 ml of MeOH. The reaction was stirred under H_2 atmosphere at room temperature for 4 hours. The reaction mixture was passed through a short column of silica gel to remove the catalyst and washed with MeOH. After removal of the solvent, the residue was dissolved in 1.5 ml of DMF and to this solution was added 0.5 ml of morpholine at 0 °C slowly. The reaction was stirred at room temperature for overnight. The solvent was evaporated in vacuo and the residue was separated by chromatography on silica gel to give 29.0 mg material which was further deacetylated in basic condition. The material got previously was dissolved in 50 ml of anhydrous THF and 5 ml of anhydrous MeOH. The solution was cooled to 0 °C and to this solution was added a solution of NaOMe (14.9 mg, 0.276 mmol) in 5 ml of anhydrous MeOH. The reaction was stirred at room temperature for overnight and quenched with 50% aq. HOAc. After evaporation of the solvent, the residue was separated by chromatography on reverse-phase silica gel to give crude product, which was further purified by gel permeation filtration on Sephadex LH-20 to give the final product **2'** (8.4 mg, 74%yield). **2'**: $[\alpha]_D^{20}$ 418.4° (c 0.1, H_2O); ^1H NMR (300MHz, CD_3OD-D_2O) δ 4.91 (d, J=3.3Hz, 1H), 4.56 (d, J=8.2Hz, 1H), 4.46 (d, J=7.4Hz, 1H), 3.52-4.22 (m, 20H), 2.10 (s, 3H), 2.06 (s, 3H), 1.36 (d, J=6.5Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD-D_2O) δ 175.90, 175.48, 104.20, 103.97, 102.47, 79.75, 78.71, 76.72, 76.56, 73.92, 73.76, 70.94, 70.52, 70.10, 69.79, 68.98, 62.25, 61.28, 56.25, 51.20, 50.79, 23.51, 19.44; HRMS(FAB) calc. for $C_{26}H_{46}N_3O_{16}$ [M+H$^+$] 688.2776, found 688.2774.

EXAMPLE 32

Preparation of thioglycoside 17': To a suspension of perbenzylated lactal **16'** (420 mg,

0.49 mmol) and 600 mg of 4Å molecular sieve in 5 ml of anhydrous CH_2Cl_2 was added benzenesulfonamide (116 mg, 0.74 mmol) at room temperature. After 10 minutes, the suspension was cooled to 0 °C and I(sym-collidine)$_2ClO_4$ was added in one portion. Fifteen minutes later, the solution was filtered through a pad of celite and washed with EtOAc. The organic solution was washed with $Na_2S_2O_3$, brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give 500 mg of iodosulfonamidate derivative (90%yield). To a solution of ethanethiol (150 μl, 1.98 mmol) in 4 ml of anhydrous DMF at -40 °C was added a solution of LiHMDS (0.88 ml, 0.88 mmol). After 15 minutes, a solution of iodosulfonamidate (450 mg, 0.397 mmol) in 6 ml of anhydrous DMF was added slowly at that temperature. The reaction mixture was stirred at -40 °C for 4 hours, and quenched with H_2O. The aqueous solution was extracted by EtOAc three times and the combined organic layer was washed with H_2O, brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give the desired thioglycoside **17'** (350 mg, 83%yield) and recover the iodosulfonamidate (60 mg). **17'**: IR (film) 3020, 3000, 2860, 1480, 1450 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J=7.7 Hz, 2H), 7.17-7.45 (m, 33H), 5.01 (d, J=8.9 Hz, 1H), 4.93 (d, J=11.4 Hz, 1H), 4.79 (s, 2H), 4.69 (m, 3H), 4.56 (d, J=11.3 Hz, 2H), 4.30-4.50 (m, 6H), 3.95 (t, J=5.0 Hz, 1H), 3.90 (d, J=2.7 Hz, 1H), 3.75 (m, 3H), 3.65 (m, 2H), 3.52 (m, 2H), 3.39-3.46 (m, 3H), 2.50 (q, J=7.4 Hz, 2H), 1.12 (t, J=7.4 Hz, 3H); HRMS (FAB) calc. for $C_{62}H_{67}O_{11}NS_2K$ [M+K$^+$] 1104.3789, found 1104.3760.

EXAMPLE 33

Preparation of trisaccharide 20': In a round-bottom flask were placed thioglycoside **17'**(2.10 g, 1.97 mmol), acceptor **18'** (964 mg, 2.95 mmol), di-*t*-butylpyridine (2.65 ml, 11.81 mmol) and 7.0 g of 4Å molecular sieve. The mixture was dissolved in 10 ml of anhydrous CH_2Cl_2 and 20 ml of anhydrous Et_2O. This solution was cooled to 0 °C and then MeOTf (1.11 ml, 8.85 mmol) was added to it slowly. The reaction mixture was stirred at 0 °C for overnight. After filtration through a pad of Celite™, the organic layer was submitted

to aqueous work-up. The EtOAc extraction was dried over Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give **20α'** (206 mg, 8%) and **20β'** (2.26 g, 86%). **20β'**: IR (film) 3020, 3000, 2860, 1480, 1450 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 7.82 (d, J=7.7 Hz, 2H), 7.20-7.45 (m, 43H), 6.32 (d, J=6.2 Hz, 1H), 4.96 (d, J=9.2 Hz, 1H), 4.90 (d, J=6.2 Hz, 1H), 4.80 (m, 4H), 4.72 (s, 2H), 4.54-4.68 (m, 6H), 4.28-4.48 (m, 6H), 4.07 (br.s, 1H), 4.00 (t, J=5.0 Hz, 1H), 3.90 (s, 1H), 3.74 (m, 4H), 3.35-3.61 (m, 10H); HRMS(FAB) calc. for $C_{80}H_{83}O_{15}NSK$ [M+K⁺] 1368.5123, found 1368.5160.

EXAMPLE 34

Preparation of trisaccharide 21': In a round-bottom flask were placed thioglycoside **17'** (966 mg, 0.906 mmol), acceptor **19'** (219 mg, 1.18 mmol), di-*t*-butylpyridine (1.22 ml, 5.44 mmol) and 2.5 g of 4Å molecular sieve. The mixture was dissolved in 5 ml of anhydrous CH_2Cl_2 and 10 ml of anhydrous Et_2O. This solution was cooled to 0 °C and then MeOTf (0.51 ml, 4.53 mmol) was added to it slowly. The reaction mixture was stirred at 0 °C for 5 hours. After filtration through a pad of Celite™, the organic layer was submitted to aqueous work-up. The EtOAc extraction was dried over Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give **21α'** (59 mg, 6%) and **21β'** (910 mg, 84%). **21α'**: IR (film) 3020, 3000, 2860, 1480, 1450 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ (7.83 (d, J=7.5 Hz, 2H), 7.12-7.46 (m, 33H), 6.36 (d, J=6.2 Hz, 1H), 5.11 (d, J=8.9 Hz, 1H), 4.98 (d, J=10.9 Hz, 1H), 4.93 (d, J=11.6, 1H), 4.83 (d, J=8.1 Hz, 1H), 4.80 (d, J=11.6 Hz, 1H), 4.68-4.73 (m, 4H), 4.50-4.58 (m, 3H), 4.27-4.32 (m, 4H), 4.27 (d, J=6.2 Hz, 1H), 4.05 (m, 1H), 3.97 (m, 2H), 3.83 (m, 2H), 3.70 (m, 2H), 3.58 (m, 2H), 3.24-3.49 (m, 4H), 1.52 (s, 3H), 1.41 (s, 3H); HRMS (FAB) calc. for $C_{69}H_{75}O_{15}NSNa$ [M+Na⁺] 1212.4756, found 1212.4720.

21β': IR (film) 3020, 3000, 2860, 1480, 1450 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ_(7.87 (d, J=7.2 Hz, 2H), 7.19-7.45 (m, 33H), 6.35 (d, J=6.2 Hz, 1H), 4.98 (d, J=8.9 Hz, 1H), 4.95 (d, J=11.6 Hz, 1H), 4.78 (m, 4H), 4.67 (m, 3H), 4.56 (m, 2H), 4.50 (d, J=12.0 Hz, 1H),

4.43 (d, J=6.2 Hz, 1H), 4.27-4.39 (m, 4H), 4.04 (d, J=6.2 Hz, 1H), 3.97 (t, J=7.2 Hz, 1H), 3.90 (d, J=2.5 Hz, 1H), 3.73-3.82 (m, 3H), 3.48-3.66 (m, 6H), 3.35-3.42 (m, 3H), 1.43 (s, 3H), 1.30 (s, 3H); HRMS (FAB) calc. for $C_{69}H_{75}O_{15}NSNa$ [M+Na$^+$] 1212.4755, found 1212.4780.

EXAMPLE 35

Preparation of trisaccharide 22': In a flame-dried flask was condensed 30 ml of anhydrous NH_3 at -78 °C. To this liquid NH_3 was added sodium metal (320 mg, 13.95 mmol) in one portion. After 15 minutes, the dry ice-ethanol bath was removed and the dark blue solution was refluxed for 20 minutes. It was cooled down to -78 °C again and a solution of trisaccharide **20'** (619 mg, 0.47 mmol) in 6 ml of anhydrous THF was added slowly. The reaction mixture was refluxed at -30 °C for half hour and quenched with 10 ml of MeOH. After evaporation of NH_3, the basic solution was neutralized by Dowex®resin. The organic solution was filtered and evaporated to give crude product which was submitted to acetylation. The crude product was dissolved in 3.0 ml of pyridine and 2.0 ml of Ac_2O in the presence of 10 mg of DMAP at 0 °C. The reaction mixture was stirred from 0 °C to room temperature for overnight. After aqueous work-up, the organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was separated by chromatography on silica gel to give peracetylated trisaccharide **22'** (233 mg, 59%). **22'**: $[\alpha]_D^{20}$ -19.77° (c 1.04, $CHCl_3$); IR(film) 1740, 1360 cm^{-1}, ^{1}H NMR (300MHz, $CDCl_3$) δ 6.46 (dd, J=6.2, 1.5 Hz, 1H), 5.64 (d, J=9.1 Hz, 1H), 5.54 (d, J=2.0Hz, 1H), 5.40 (d, J=4.5 Hz, 1H), 5.36 (d, J=2.9 Hz, 1H), 5.12 (m, 2H), 4.98 (dd, J=10.4, 3.4 Hz, 1H), 4.70 (d, J=6.2 Hz, 1H), 4.58 (d, J=7.3 Hz, 1H), 4.50 (m, 2H), 4.26 (t, J=5.0 Hz, 1H), 4.12 (m, 3H), 3.89 (m, 2H), 3.78 (m, 2H), 3.64 (m, 1H), 2.16 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.29, 170.14, 169.24, 145.34, 128.20, 100.85, 100.72, 88.86, 75.58, 74.26, 72.58, 72.06, 70.71, 70.61, 68.98, 66.77, 66.55, 64.19, 63.53, 62.09, 60.70, 52.97, 23.05, 20.72, 20.56; HRMS (FAB) calc. for $C_{36}H_{49}O_{22}NNa$ [M+Na$^+$] 870.2645, found 870.2644.

EXAMPLE 36

Preparation of trisaccharide donor 23': To a solution of trisaccharide glycal **20'** (460 mg, 0.346 mmol) in 3 ml of anhydrous CH_3CN at -25 °C were added NaN_3 (34 mg, 0.519 mmol) and CAN (569 mg, 1 .4 mmol) subsequently. The mixture was stirred at -25 °C for 8 hours. After aqueous work-up, the organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was separated by chromatography on silica gel to give a mixture of azidonitrate derivatives (134 mg, 27%). This azidonitrate mixture was hydrolyzed in the reductive condition. The azidonitrates was dissolved in 2 ml of anhydrous CH_3CN at room temperature. $EtN(i\text{-}Pr)_2$ (16 μl, 0.091 mmol) and PhSH (28 μl, 0.272 mmol) were added subsequently. After 15 minutes, the reaction was complete and the solvent was evaporated at room temperature. The hemiacetal derivative (103 mg, 74%) was obtained after chromatography on silica gel. This hemiacetal (95 mg, 0.068 mmol) was dissolved in 2 ml of anhydrous CH_2Cl_2. To this solution were added 1 ml of CCl_3CN and 0.5 g of K_2CO_3 at room temperature. The reaction was run for overnight. After filtration through a pad of Celite™, the organic solvent was evaporated and the residue was separated by chromatography on silica gel to give **23α'** (18 mg, 17%) and **23β'** (70 mg, 67%). **23α'**: ^{1}H NMR (300MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.96 (d, J=8.2 Hz, 2H), 6.92-7.50 m, 33H), 6.56 (d, J=2.8 Hz, 1H), 5.02 (m, 3H), 4.92 (d, J=11.6 Hz, 2H), 4.86 (d, J=11.6 lz, 1H), 4.22-4.64 (m, 18H), 3.95-4.07 (m, 3H), 3.85 (m, 2H), 3.72 (m, 2H), 3.63 (m, 1H), 35-3.56 (m, 4H), 3.34 (dd, J=10.3, 2.8 Hz, 1H).

β': ^{1}H NMR (300MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.10 (d, J=8.1 Hz, 2H), 6.90-7.45 (m, 1), 6.37 (d, J=9.4Hz, 1H), 5.93 (d, J=8.2 Hz, 1H), 5.04 (d, J=11.6 Hz, 2H), 4.98 (d, 1.6 Hz, 1H), 4.90 (d, J=11.7 Hz, 1H), 4.83 (d, J=11.7 Hz, 1H), 4.79 (d, J=11.6 Hz, 4.77 (d, j=11.6 Hz, 1H), 4.72 (d, J=8.2 Hz, 1H), 4.40-4.63 (m, 8H), 4.19-4.38 (m, 3.86-4.10 (m, 6H), 3.63 (m, 2H), 3.42-3.50 (m, 4H), 3.35 (m, 2H), 3.25 (d, J=9.1 Hz,

EXAMPLE 37

Preparation of trisaccharide donor 24': To a solution of trisaccharide glycal **21'** (225 mg, 0.264 mmol) in 2 ml of anhydrous CH_3CN at -15 °C were added NaN_3 (26 mg, 0.40 mmol) and CAN (436 mg, 0.794 mmol) subsequently. The mixture was stirred at -15 °C for overnight. After aqueous work-up, the organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was separated by chromatography on silica gel to give a mixture of azidonitrate derivatives (130 mg, 51%). This azidonitrate mixture was hydrolyzed in the reductive condition. The azidonitrates (125 mg, 0.129 mmol) was dissolved in 5 ml of anhydrous CH_3CN at room temperature. EtN(*i*-Pr)$_2$ (25 μl, 0.147 mmol) and PhSH (45 μl, 0.441 mmol) were added subsequently. After 15 minutes, the reaction was complete and the solvent was evaporated at room temperature. The hemiacetal derivative (92 mg, 77%) was obtained after chromatography on silica gel. This hemiacetal (80 mg, 0.087 mmol) was dissolved in 5 ml of anhydrous CH_2Cl_2. To this solution were added 0.9 ml of CCl_3CN and 0.12 g of K_2CO_3 at room temperature. The reaction was run for overnight. After filtration through a pad of Celite™, the organic solvent was evaporated and the residue was separated by chromatography on silica gel to give a mixture of α and β isomer of **24'** (71 mg, 77%, α:β 3:1). **24'**: ^{1}H NMR (300MHz, $CDCl_3$) δ 9.55 (s, 1H, NH of β isomer), 8.71 (s, 1H, NH of α isomer), 6.54 (d, J = 3.6 Hz, amomeric H of α isomer)

EXAMPLE 38

Preparation of trisaccharide donor 25': The azidonitrate derivatives (100 mg, 0.103 mmol) from peracetylated trisaccharide **21'** was dissolved in 0.5 ml of anhydrous CH_3CN at room temperature. To this solution was added anhydrous LiBr (45 mg, 0.52 mmol). The mixture was stirred for 3 hours. After aqueous work-up, the solvent was evaporated and the residue was separated by chromatography on silica gel to give compound **25'** (91 mg, 90%). **25'**: ^{1}H NMR (300MHz, $CDCl_3$) δ 6.04 (d, J = 3.6 Hz, 1H, anomeric H).

EXAMPLE 39

Preparation of trisaccharide donor 26': The trisaccharide donor **25'** (91 mg, 0.093 mmol)

was dissolved in 2 ml of anhydrous THF at 0 °C. To this solution was added LiSPh (100 ml, 0.103mmol). The reaction was run at 0 °C for half hour. The solvent was removed and the residue was separated by chromatography on silica gel to give compound **26'** (61 mg, 66%). **26'**: IR (film) 3000, 2100, 1750, 1680, 1500 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 7.61 (m, 2H), 7.39 (m, 3H), 5.50 (d, J=9.1 Hz, 1H), 5.35 (m, 2H), 5.11 (m, 2H), 4.96 (dt, J=10.5, 3.5 Hz, 1H), 4.84 (dd, J=10.2, 3.0 Hz, 1H), 4.50 (m, 4H), 4.16 (m, 3H), 3.59-3.90 (m, 8H), 2.15 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 6H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H), 1.87 (s, 3H).

EXAMPLE 40

Preparation of trisaccharide donor 27': The trisaccharide **21'** (860 mg, 0.722 mmol) was dissolved in 2 ml of pyridine and 1 ml of Ac_2O in the presence of 10 mg of DMAP. The reaction was run at 0 °C to room temperature for overnight. After aqueous work-up, the solvent was removed and the residue was dissolved in 10 ml of MeOH and 5 ml of EtOAc at room temperature. To this solution were added Na_2HPO_4 (410 mg, 2.89 mmol) and 20% Na-Hg (1.0 g, 4.35 mmol). The reaction was run for 2 hours and aqueous work-up followed. After removal of the organic solvent, the residue was separated by chromatography on silica gel to give *N*-acetyl trisaccharide glycal (740 mg, 94%). The trisaccharide glycal (624 mg, 0.571 mmol) was dissolved in 3 ml of anhydrous CH_3CN at -40 °C. To the solution were added NaN_3 (56 mg, 0.86 mmol) and CAN (939 mg, 1.71 mmol) subsequently. The mixture was stirred at -40 °C for 4 hours. After aqueous work-up, the organic solvent was removed and the residue was separated by chromatography on silica gel to give a mixture of α and β azidonitrate anomers (191 mg, 27%). This mixture of anomers (172 mg, 0.137 mmol) was dissolved in 1 ml of CH_3CN at room temperature. To the solution were added $EtN(i\text{-}Pr)_2$ (24 μl, 0.137 mmol) and PhSH (42 μl, 0.410 mmol) subsequently. The reaction was complete in half hour and the solvent was blown off. Separation on column afforded desired hemiacetal (170 mg). This hemiacetal was dissolved in 1 ml of CH_2Cl_2 at room temperature. To the solution were added 1 ml of

CCl_3CN and 500 mg of K_2CO_3. The reaction was run at room temperature for overnight. After filtration through a pad of celite, the organic solvent was removed and the residue was separated by chromatography on silica gel to give desired α-trichloroacetimidate **27'** (70 mg, 42%). **27'**: IR (film) 3000, 2120, 1670, 1490, 1450 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.06-7.48 (m, 30H), 6.44 (d, J=3.0 Hz, 1H), 5.21 (d, J=11.4 Hz, 1H), 5.03 (m, 2H), 4.89 (d, J=11.0 Hz, 1H), 4.80 (d, J=11.3 Hz, 1H), 4.69 (d, J=11.1Hz, 1H), 4.64 (d, J=7.8 Hz, 1H), 4.44-4.58 (m, 5H), 4.18-4.36 (m, 7H), 3.96-4.08 (m, 3H), 3.72-3.81 (m, 3H), 3.38-3.62 (m, 6H), 3.31 (dd, J=7.0, 2.7 Hz, 1H), 1.59 (s, 3H), 1.31 (s, 3H), 1.14 (s, 3H); HRMS (FAB) calc. for $C_{68}H_{74}O_{15}N_5Cl_3Na$ [M+Na+] 1316.4145, found 1316.4110.

EXAMPLE 41

Coupling of trisaccharide donor 23α' with methyl N-Fmoc Serinate: To a solution of trisaccharide donor **23α'** (70 mg, 0.046 mmol), methyl N-Fmoc serinate (23.4 mg, 0.068 mmol) and 300 mg of 4Å molecular sieve in 0.5 ml of THF at -78 °C was added TMSOTf (4.6 μl, 0.023 mmol). The reaction was stirred at -35 °C for overnight. The reaction was quenched by Et_3N and the solution was filtered through a pad of celite. The filtrate was evaporated and the residue was separated by chromatography on silica gel to give **29α'** (70 mg, 90%) and **29β'** (7.0 mg, 9.0%).

EXAMPLE 42

Coupling of trisaccharide donor 24' with benzyl N-Fmoc serinate: To a solution of trisaccharide donor **24'** (33 mg, 0.030 mmol), benzyl N-Fmoc serinate (33.0 mg, 0.075 mmol) and 100 mg of 4Å molecular sieve in 0.3 ml of THF at -78 °C was added TMSOTf (6.0 μl, 0.030 mmol). The reaction was stirred from -78 °C to room temperature for 2 hours. The reaction was quenched by Et_3N and the solution was filtered through a pad of celite. The filtrate was evaporated and the residue was separated by chromatography on silica gel to give **30'** (8.6 mg, 22%, α:β 2:1). **30'**: IR (film) 3400, 3000, 2100, 1740, 1500 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 6.25 (d, J=8.4 Hz, 2/3H), 5.90 (d, J=8.6 Hz, 1/3H),

5.76 (d, J=9.0 Hz, 1/3H), 5.71 (d, J=9.0 Hz, 2/3); MS(CI) 1306 [M^+].

EXAMPLE 43

Coupling of trisaccharide donor 25α' with benzyl N-Fmoc serinate: To a solution of benzyl N-Fmoc serinate (45 mg, 0.107 mmol), $AgClO_4$ (37.0 mg, 0.179 mmol) and 200 mg of 4Å molecular sieve in 0.6 ml of anhydrous CH_2Cl_2 was added a solution of trisaccharide donor **25α'** (88 mg, 0.0893 mmol) in 0.5 ml of CH_2Cl_2 slowly. The reaction was run at room temperature for overnight. After filtration through a pad of celite, the solvent was removed and the residue was separated by chromatography on silica gel to give the coupling product **30'** (66 mg, 56%, α:β 3.5 :1).

EXAMPLE 44

Coupling of trisaccharide donor 26β' with benzyl N-Fmoc serinate: To a solution of benzyl N-Fmoc serinate (45 mg, 0.107 mmol), trisaccharide donor **26β'** (23 mg, 0.023 mmol) and 50 mg of 4Å molecular sieve in 1.0 ml of anhydrous CH_2Cl_2 at 0 °C was added a solution of NIS (6.2 mg, 0.027 mmol) and TfOH (0.24 μl, 0.003 mmol) in 0.5 ml of CH_2Cl_2 slowly. The reaction was run at 0 0C for 1 hour. The reaction was quenched by Et_3N and aqueous work-up followed. The organic solvent was dried over Na_2SO_4. After removal of the solvent, the residue was separated by chromatography on silica gel to give the coupling product **30'** (12.1 mg, 40%, α:β 2 :1).

EXAMPLE 45

Coupling of trisaccharide donor 27α' with benzyl N-Fmoc serinate: To a solution of trisaccharide donor **27α'** (40.1 mg, 0.029 mmol), benzyl N-Fmoc serinate (18.0 mg, 0.044 mmol) and 200 mg of 4Å molecular sieve in 2.0 ml of THF at -20 °C was added TMSOTf (1.8 μl, 0.009 mmol). The reaction was stirred from -20 °C to room temperature for 3 hours. The reaction was quenched by Et_3N and aqueous work-up followed. After dried over Na_2SO_4, the filtrate was evaporated and the residue was separated by chromatography on

silica gel to give **31'** (24 mg, 51%). **31'**: IR(film) 3000, 2920, 2860, 2100, 1720, 1665, 1500, 1480, 1450 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 7.78 (m, 2H), 7.65 (d, J=7.5 Hz, 1H), 7.60 (d, J=7.5 Hz, 1H), 7.20-7.42 (m, 39 H), 6.18 (d, J=7.8 Hz, 1H), 6.05 (d, J=7.3 Hz, 1H), 5.23 (s, 2H), 4.95-5.02 (m, 3H), 4.80 (s, 2H), 4.78 (d, J=2.8 Hz, 1H, anomeric H), 4.72 (s, 2H), 4.58 (m, 4H), 4.37-4.52 (m, 6H), 4.24-4.31 (m, 2H), 4.20 (m, 1H), 4.08 (m, 2H), 3.92-4.02 (m, 5H), 3.78-3.85 (m, 5H), 3.65 (m, 1H), 3.58 (t, J=6.2Hz, 1H), 3.36-3.46 (m, 5H), 3.26 (dd, J=7.5, 2.8 Hz, 1H), 1.85 (s, 3H), 1.48 (s, 3H), 1.34 (S, 3H); HRMS (FAB) calc. for $C_{90}H_{95}O_{19}N_5Na$ [M+Na+] 1572.6520, found 1572.6550.

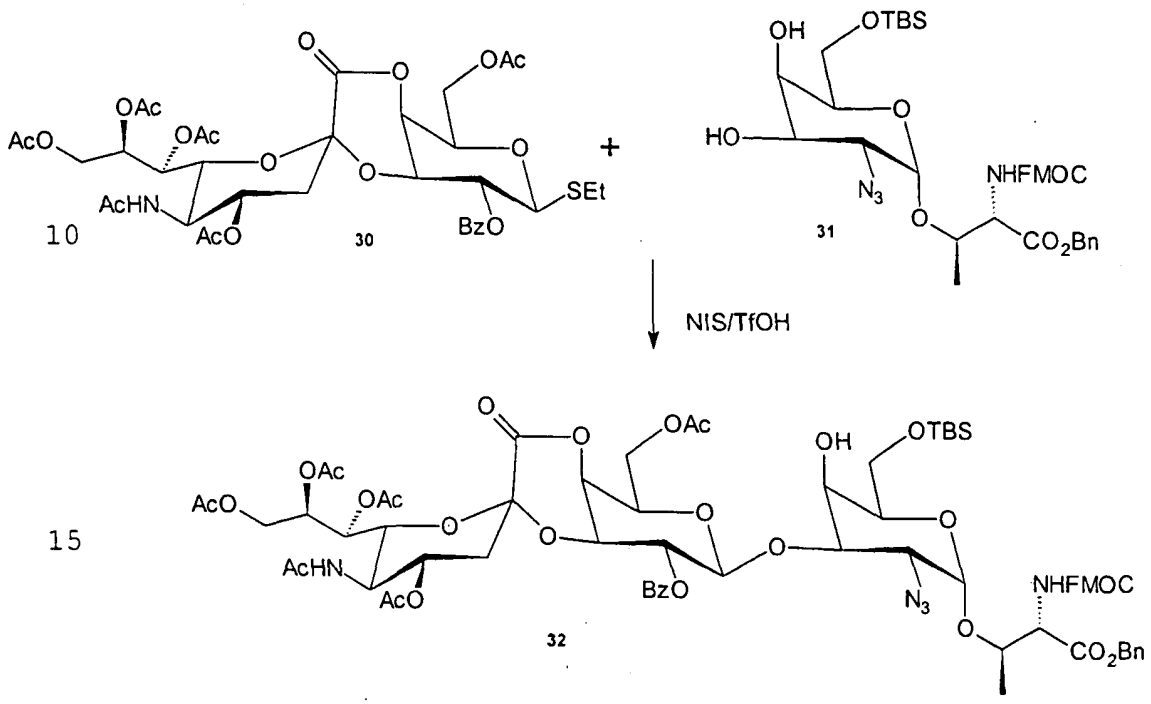
EXAMPLE 46

Coupling of trisaccharide donor 28' with benzyl N-Fmoc serinate: To a solution of trisaccharide donor **28'** (α:β 1:1)(162 mg, 0.163 mmol), benzyl N-Fmoc serinate (48.0 mg, 0.097 mmol) and 300 mg of 4Å molecular sieve in 2.0 ml of THF at -78 °C was added BF_3Et_2O (0.5 eq., 0.082 mmol) in CH_2Cl_2. The reaction was stirred from -78 °C to room temperature for 2 hours. The reaction was quenched by Et_3N and aqueous work-up followed. After dried over Na_2SO_4, the filtrate was evaporated and the residue was separated by chromatography on silica gel to give **32'** (81 mg, 67%). **32'**: IR(film) 3420, 3020, 2940, 2880, 2120, 1745, 1500, 1450 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J=7.4 Hz, 2H), 7.60 (t, J=7.5 Hz, 2H), 7.20-7.39 (m, 9H), 5.85 (d, J=8.4 Hz, 1H), 5.48 (d, J=12.6 Hz, 1H), 5.32 (d, J=3.4 Hz, 1H), 5.19 (d, J=12.6 Hz, 1 H), 5.07 (d, J=8.0 Hz, 1H), 4.90 (dd, J=10.3, 3.4 Hz, 1H), 4,83 (t, J=10.3 Hz, 1H), 4.72 (d, J=9.3 Hz, 1H), 4.67 (d, J=9.6 Hz, 1H), 3.80-4.47 (m, 9H), 3.62 (t, J=9.5 Hz, 1H), 3.32-3.42 (m, 2H), 2.93 (d, J=7.7 Hz, 1H), 2.14 (s, 3H), 2.08 (s, 6H), 2.04 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H).

EXAMPLE 47

Coupling of trisaccharide donor 28β' with benzyl N-Fmoc serinate: To a solution of trisaccharide donor **28β'** (12.0 mg, 0.012 mmol), benzyl N-Fmoc serinate (9.0 mg, 0.022

mmol) and 100 mg of 4Å molecular sieve in 0.5 ml of THF at -40 °C was added $BF_3{\cdot}Et_2O$ (1.5 eq. , 0.018 mmol) in CH_2Cl_2. The reaction was stirred from -40 °C to room temperature for 2 hours. The reaction was quenched by Et_3N and aqueous work-up followed. After dried over Na_2SO_4, the filtrate was evaporated and the residue was separated by chromatography on silica gel to give **32'** (5.2 mg, 35%).

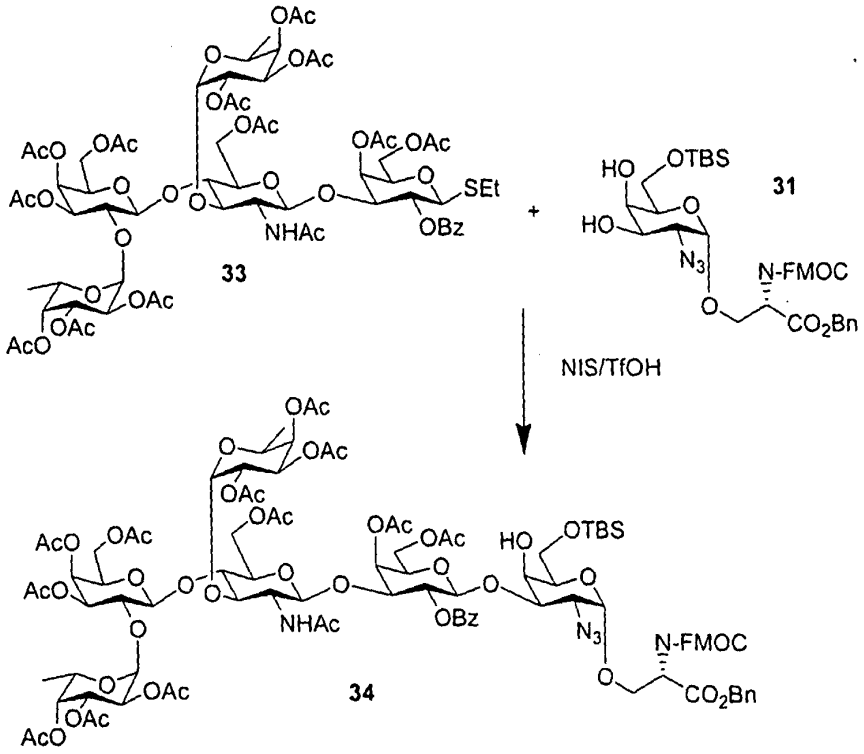


2,3-ST Antigen Precursor

A mixture of thioethyl glycosyl donor **30** (52 mg, 0.064 mmol) and 6-TBDMS acceptor **31** (94 mg, 0.13 mmol) were azeotroped with benzene (4 x 50 mL), then placed under high vacuum for 1 h. The mixture was placed under nitrogen, at which time 4Å mol sieves (0.5 g), CH_2Cl_2 (5 mL), and NIS (36 mg, 0.16 mmol) were added. The mixture was cooled to 0 °C, and trifluoromethanesulfonic acid (1% in CH_2Cl_2, 0.96 mL, 0.064 mmol) was added

dropwise over 5 min. The suspension was warmed to ambient temperature immediately following addition and stirred 20 min. The mixture was partitioned between EtOAc (50 mL) and sat. $NaHCO_3$ (50 mL). The phases were separated, and the organic phase washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on silica gel (4:1, EtOAc:hexanes) to provide 59 mg (62%) of the trisaccharide **32** as a colorless crystalline solid.

Trisaccharide **32**: $[\alpha]_D^{23}$ +29.6 (c 1.65, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02 (d, *J* = 7.3 Hz, 2H), 7.77 (d, *J* = 7.7 Hz, 2H), 7.56 (m, 2H), 7.26-7.50 (m, 12H), 5.59 (d, *J* = 9.5 Hz, 1H), 5.51 (ddd, *J* = 15.9, 11.2, 5.5 Hz, 1H), 5.59 (d, *J* = 9.5 Hz, 1H), 5.21 (br s, 4H), 5.07 (m, 3H), 4.85 (d, *J* = 8.0 Hz, 1H), 4.66 (m, 2H), 4.19-4.48 (m, 10H), 4.13 (br s, 1H), 4.66 (m, 2H), 4.19-4.48 (m, 10H), 4.13 (br s, 1H), 4.09 (d, *J* = 10.4 Hz, 1H), 4.04 (m, 1H), 3.94 (m, 3H), 3.78 (m, 4H), 3.64 (d, *J* = 10.4 Hz, 1H), 3.45 (dd, *J* = 10.5, 3.9 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.00 (s,3H), 1.99 (s, 3H), 1.86 (s, 3H), 1.78 (m, 1H), 1.29 (d, *J* = 6.3 Hz, 3H), 0.86 (s, 9H) 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.95, 170.66, 170.39, 169.95, 165.30, 163.02, 156.70, 143.92, 143.63, 141.24, 134.81, 133.41, 129.74, 129.11, 128.58, 128.54, 128.49, 128.36, 128.01, 127.71, 127.09, 127.02, 125.17, 125.11, 119.96, 100.80, 99.49, 95.16, 78.46, 76.17, 72.78, 72.14, 71.75, 71.54, 71.25, 70.92, 70.05, 69.18, 68.57, 68.33, 67.61, 67.33, 67.07, 63.05, 62.25, 62.21, 58.79, 58.70, 49.23, 47.11, 37.97, 25.83, 23.10, 20.82, 20.73, 20.71, 20.63, 20.55, 18.78, 18.28, 18.00, 17.88, 17.84, 11.89, -5.35, -5.50; IR (neat): 2953, 2931, 2111, 1744, 1689 cm^{-1}. HRMS: Calcd for $C_{72}H_{87}N_5O_{27}SiNa$: 1504.5255; Found: 1504.5202.



Le^y Antigen Precursor

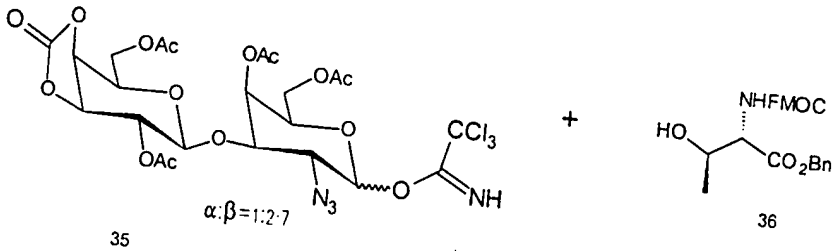
To thiodonor **33** (44.0 mg, 29.5 μmol) and acceptor **31** (42.4 mg, 59.0 μmol) (azeotroped 3 times with toluene) were added CH_2Cl_2 and freshly activated 4Å molecular sieves. The mixture was stirred for 20 min, then cooled to 0°C. N-iodosuccinimide (16.6 mg, 73.8 μmol) was added, followed by the dropwise addition of a 1% solution of TfOH in CH_2Cl_2. The red mixture was stirred at 0°C for 5 min, then was diluted with EtOAc. The organic phase was washed with sat. $NaHCO_3$, sat. $Na_2S_2O_3$, and brine, dried over $MgSO_4$, then concentrated *in vacuo*. Flash chromatography (1:1 EtOAc/CH_2Cl_2 to 2:1 EtOAc/CH_2Cl_2) afforded 43.2 mg (68%) of the coupled product **34**.

Data for Hexasaccharide 34: $[\alpha]_D^{23}$ –26.4 (c 1.00, $CHCl_3$); ^{1}HNMR ($CDCl_3$) δ 8.10 (d, *J* = 7.4 Hz, 2H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.54 (t, *J* = 7.2 Hz,

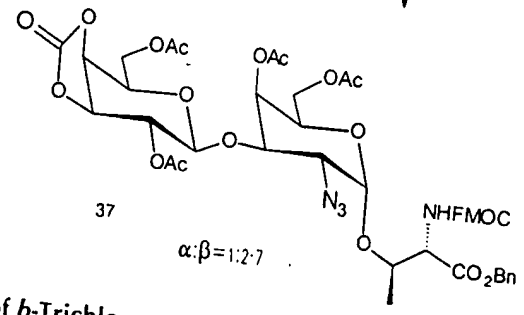
1H), 7.43-7.24 (m, 12H), 5.86 (d, J = 8.5 Hz, 1H), 5.52-5.47 (m, 2H), 5.35-5.32 (m, 4H), 5.18-5.05 (m, 5H), 5.04-4.98 (m, 3H), 4.95-4.88 (m, 3H), 4.80 (d, J = 7.9 Hz, 1H), 4.72 (d, J = 3.3 Hz, 1H), 4.59-4.56 (m, 2H), 4.51 (dd, J = 11.7, 5.7 Hz, 1H), 4.43-4.37 (m, 2H), 4.33-4.23 (m, 2H), 4.21-4.07 (m, 6H), 4.03-3.84 (m, 5H), 3.80-3.73 (m, 4H), 3.44 (d, J = 10.3 Hz, 1H), 3.43 (d, J = 10.5 Hz, 1H), 3.21-3.13 (m, 1H), 2.83 (s, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.99 (s, 6H), 1.27 (s, 3H), 1.14 (d, J = 5.6 Hz, 6H), 0.86 (s, 9H), 0.04 (s, 6H); ^{13}CNMR ($CDCl_3$) δ 171.37, 171.23, 171.10, 170.96, 170.91, 170.87, 170.85, 170.74, 170.54, 170.39, 170.17, 169.96, 169.92, 165.79, 156.31, 144.18, 141.69, 135.43, 134.09, 130.24, 129.51, 129.05, 129.01, 128.92, 128.84, 128.17, 127.50, 125.58, 125.54, 120.43, 102.39, 100.83, 100.69, 99.87, 96.62, 96.09, 78.11, 77.30, 74.25, 73.76, 73.52, 73.30, 72.96, 72.04, 71.81, 71.33, 71.26, 71.10, 71.03, 69.81, 69.38, 68.71, 68.61, 68.23, 68.10, 67.99, 67.95, 67.67, 67.29, 65.45, 64.36, 62.95, 62.20, 60.95, 58.84, 58.76, 54.87, 47.51, 26.25, 22.97, 21.47, 21.30, 21.26, 21.14, 21.08, 21.05, 20.99, 18.69, 16.28, 15.99, -4.98, -5.07; IR (neat): 2935, 2110, 1746 cm^{-1}. HRMS: Calcd for CHNOSi: ; Found.

Experimental for Figure 12: Sialylated acceptor (58 mg, 0.054 mmol) and thioglycoside (22 mg, 0.027 mmol) were azeotroped with benzene (3 x 5 mL). NIS (15.2 mg, 0.068 mmol), 0.1 g of 4Å mol sieves, and 2.0 mL of CH_2Cl_2 were then added. A freshly prepared solution of triflic acid (1% soln in CH_2Cl_2, 0.24 mL) was then added dropwise. After 5 min, the reaction was judged complete by TLC and quenched with triethylamine. Flash chromatography (3→3.5→4→4.5→5% MeOH in CH_2Cl_2) afforded 26 mg (53%) of the tetrasaccharide as a white film: $[\alpha]_D^{23}$ +20.8 (c = 1.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 6.7 Hz, 2H), 7.77 (d, J = 6.7 Hz, 2H), 7.60 (t, J = 6.8 Hz, 2H), 7.53 (t, J = 7.2 Hz, 1H), 7.04-7.44 (m, 11H), 5.84 (d, J = 8.3 Hz, 1H), 5.51 (dt, J = 10.7, 5.4 Hz, 1H), 5.16-5.38 (m, 10H), 5.06 (bs, 1H), 4.85 (bm, 1H), 4.77 (d, J = 7.9 Hz, 1H), 4.75 (bs, 1H), 4.61 (bd, J = 8.3 Hz, 2H), 3.75-4.48 (m, 22H), 3.65 (d, J = 10.5 Hz, 1H), 3.55 (dd, J = 9.7, 5.8 Hz, 1H), 3.48 (dd, J = 10.4, 3.4 Hz, 1H), 2.61 (bs, 1H), 2.56 (dd, J = 12.8, 4.6 Hz, 1H),

2.51 (dd, J = 13.9, 5.5 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.87 (s, 3H), 1.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.9, 170.7, 170.6, 170.4, 170.3, 170.2, 170.0, 169.9, 169.8, 168.0, 165.3, 163.0, 155.8, 143.8, 143.7, 141.2, 135.0, 133.4, 129.7, 129.1, 128.6, 128.5, 128.4, 128.3, 127.8, 127.1, 125.2, 120.0, 100.8, 99.0, 98.7, 95.1, 72.8, 72.7, 72.2, 71.2, 69.4, 69.2, 69.0, 68.9, 68.8, 68.0, 67.7, 67.6, 67.2, 67.0, 66.3, 62.5, 62.0, 58.3, 54.4, 53.4, 52.8, 49.3, 47.1, 38.0, 37.5, 29.7, 23.1, 23.0, 21.0, 20.8, 20.7, 20.6, 20.5; IR (film) 3366, 3065, 2959, 2111, 1744, 1687, 1533, 1369, 1225 cm^{-1}. FAB HRMS *m/e* calcd for (M+ Na) $C_{85}H_{98}N_6O_{39}Na$ 1849.5767, found 1849.5766.



35 36



37

Coupling of *b*-Trichloroacetimidate with Protected Threonine

To a solution of trichloroacetimidate **35** (98 mg, 0.13 mmol), threonine derivative **36** (70 mg, 0.167 mmol) and 100 mg 4Å molecular sieve in 6 ml of anhydrous CH_2Cl_2 at -30°C was added TMSOTf (14 mL, 0.07 mmol). The reaction was stirred at -30°C for 1 hour, then neutralized with Et_3N. The reaction mixture was filtered through a pad of Celite™ and washed with EtOAc. The filtrate was washed with H_2O, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was separated by chromatography on silica gel to give β-product **37β** (56 mg, 42%) and the α-product **37α** (57 mg, 42%).

Discussion

The synthetic approach taken in the present invention encompasses four phases (Figure 2). First, the complete glycodomain is assembled in the form of an advanced glycal. This is followed by efficient coupling to a serine, threonine or analogous residue. The third stage involves peptide assembly incorporating the full glycosyl domain amino acids into the peptide backbone. The concluding phase involves global deprotection either in concurrent or segmental modes.

The synthetic starting point was the readily available glycal **2** (Figure 3). (Oxidation of this compound with dimethyldioxirane and subsequent coupling of the resultant epoxide with 6-O-TIPS-galactal was promoted by $ZnCl_2$ in the standard way. Toyokuni, T.; Singhal, A.K.; *Chem. Soc. Rev.* **1995**, *24*, 231. Acetylation of the crude product yielded disaccharide **3** in high yield and stereoselectivity. Removal of the TIPS protecting group under mild conditions set the stage for attachment of sialic acid to acceptor **4**. The use of sialyl phosphite **5** as the donor, under promotion of catalytic amounts of TMSOTf, consistently provided high yields (80 - 85%) of a 4:1 mixture of products. Martin, T.J., *et al., Glycoconjugate J.* **1993**, *10*, 16. Sim, M.M, *et al., J. Am. Chem. Soc.* **1993**, *115*, 2260. Thus, the advanced glycal **6** ("2,6-ST glycal") is available in four steps with high efficiency.

The trisaccharide glycal **6** was submitted to azidonitration as shown (Figure 3). Compound **7** thus obtained in 60% yield lent itself to conversion to a variety of donor constructs (see **8 - 11**). For instance, α-bromide **8** can be used as a donor directly or could be converted to β-phenylthioglycoside **11** with lithium thiophenoxide in a stereoselective manner. Alternatively, mixtures of nitrates **7** was hydrolyzed and the resulting hemiacetal converted to 1:1 mixture of α:β trichloroacetamidates **(9)** and diethylphoshites **(10)** in high yields (Figure 3). (Nitrate hydrolysis: Gauffeny, F., *et al., Carbohydr. Chem.* **1991**, 219, 237. Preparation and application of trichloroacetamidates: Schmidt, R.R. and Kinzy, W.; *Adv. Carbohydr. Chem. Biochem.* **1994**, 50, 21. Phosphite donors: Kondo, H., *et al.; J. Org. Chem.* **1994**, *59*, 864.)

Table I. Reaction of **11** with N-FMOC-Ser(OH)-OBn.

X (11)	Catalyst/Promoter	R = H (12) α:β(%)	R = CH_3 (13) α:β(%)
- Br (8α)	$AgClO_4$(1.5eq), CH_2Cl_2, rt	2.6 : 1 (70%)	α only (74%)
- $O(CNH)CCl_3$ (9β)	BF_3OEt_2(0.5eq), THF, -30C	12 : 1 (65%)	α only (63%)
-$O(CNH)CCl_3$ (9αβ 1:1)	BF_3OEt_2(0.5eq), THF, -30C	4 : 1 (66%)	α only (60%)
- $OP(OEt)_2$ (10αβ 1:1)	BF_3OEt_2(0.5eq), THF, -30C	30 : 1 (30%)	—

The availability of various donor types **(8-11)** enabled the investigation of the direct coupling of (2,6)-ST trisaccharide to benzyl ester of N-Fmoc-protected L-serine and L-threonine. The results are summarized in Table 1. As with Fmoc protected L-threonine as the acceptor, all of the donors afforded the α-O glycosyl threonine system in high stereoselectivity. By contrast, the outcome of the coupling reactions with similarly protected L-serine acceptors was dependent on the character of the donor and on the reaction conditions. In all cases, the desired α-anomer **12** was the major product. (For previous attempts to couple a trisaccharide donor to serine, in which β-anomers were isolated as the major products, see: Paulsen, H. *et al., Liebigs Ann. Chem.* **1988**, 75; Iijima, H.; Ogawa, T., *Carbohydr. Res.* **1989**, 186, 95.) With donor **10** the ratio of desired α-product:undesired β-glycoside was ca 30:1.

The glycopeptide assembly phase was entered with building units **14** and **15**, thereby reducing the number of required chemical operations to be performed on the final glycopeptide. Thus, compounds **14** and **15** were obtained in two steps from **12** and **13**, respectively. The azide functionality was transformed directly to N-acetyl groups by the action of CH_3COSH in 78-80% yield and the benzyl ester was removed quantitatively

by hydrogenolysis (Figure 4). Paulsen, H., et *al.*, *Liebigs Ann. Chem.* **1994**, 381.

The glycopeptide backbone was built in the C→N-terminus direction (Figure 4). Iteration of the coupling step between the N-terminus of a peptide and protected glycosyl amino acid, followed by removal of the FMOC protecting group provided protected pentapeptide **16**. The peptide coupling steps of block structures such as **12** and **13** proceeded in excellent yields. Both IIDQ and DICD coupling reagents work well (85-90%). FMOC deprotection was achieved under mild treatment with KF in DMF in the presence of 18-crown-6. Jiang, J., et *al.*, *Synth. Commun.* **1994**, *24*, 187. The binal deblocking of glycopeptide **16** was accomplished in three stages: (i) Fmoc removal with KF and protection of the amino terminus with acetyl group; (ii) hydrogenolysis of the benzyl ester; and (iii) final saponification of three methyl esters, cyclic carbonates and acetyl protection with aqueous NaOH leading to glycopeptide mucin model **1** (Figure 4).

The orthogonal exposure of both N- and C-termini provided an opportunity for further extension of the glycopeptide constructs *via* fragment joining. In order to demonstrate the viability of such claims, a nonapeptide with ST triad **19** was made by means of coupling tripeptide **18** to hexapeptide **17** (see Figure 5). The previous deprotection protocol provided nonapeptide mucin model **20**, wherein the *o*-glycosylated serine-threonine triad had been incorporated in the middle of the peptide.

Vaccination with Tn Cluster Constructs in Mice

The present invention provides anti-tumor vaccines wherein the glycopeptide antigen disclosed herein is attached to the lipopeptide carrier PamCys. The conjugation of the antigen to the new carrier represents a major simplification in comparison to traditional protein carriers. Tables 2 and 3 compare the immunogenicity of the new constructs with the protein carrier vaccines in mice. These novel constructs proved immunogenic in mice. As shown in the Tables, the Tn-PamCys constructs elicit high titers of both IgM and IgG after the third vaccination of mice. Even higher titers are induced after the fifth vaccination. The Tn-KLH vaccine yields stronger overall response.

However, the relative ratio of IgM/IgG differs between the two vaccines. Tn-KLH gives higher IgM/IgG ratio than the Tn Pamcys. In a relative sense, the novel Tn-PamCys vaccine elicits a stronger IgG response. In contrast to protein carrier vaccines, the adjuvant QS-21 does not provide any additional enhancement of immunogenicity. Accordingly, the PamCys lipopeptide carrier may be considered as a "built-in" immunostimulant/adjuvant. Furthermore, it should be noted that QS-21 enhances the IgM response to Tn-PamCys at the expense of IgG titers. A vaccine based on PamCys carriers is targeted against prostate tumors.

Table 2. Antibody Titers by Elisa against Tn-Cluster: 10 μg Tn cluster-Pam

	Pre-serum		10 days post 3rd	
Group	IgM	IgG	IgM	IgG
1.1	50	0	450	450
1.2	50	0	1350	50
1.3	50	0	4050	150
1.4	0	0	4050	150
1.5	0	0	450	1350
10 μg Tn cluster-pam + QS-21				
2.1	50	0	1250	50
2.2	0	0	1350	0
2.3	0	0	1350	50
2.4	0	0	1350	150
2.5	50	0	1350	150
3 μg Tn cluster KLH + QS-21				
3.1	0	0	12150	450
3.2	0	0	12150	4050
3.3	0	0	36450	450
3.4	0	0	36450	450

3.5	0	0	36450	1350
3 ug Tn cluster BSA + QS-21				
4.1	0	0	450	1350
4.2	0	0	150	4050
4.3	0	50	450	450
4.4	0	0	450	150
4.5	0	0	1350	150

0.3 μg/well antigen plated in alcohol; serum drawn 11 days post 3rd vaccine.

Table 3. Antibody Titers by Elisa against Tn-Cluster: Tn Cluster-Pam

	Pre-serum (before 5th Vaccination)		Post Serum (10 days after 5th Vaccination)	
Group	IgM	IgG	IgM	IgG
1.1	2560	200	640	5120
1.2	25.600	800	1280	320
1.3	640	160	640	1280
1.4	2560	1280	25.600	5120
1.5	640	5120	2560	5120
Tn Cluster-Pam + QS-21				
2.1	6400	1280	128.0000	
2.2	3200	160	5120	200
2.3	3200	1280	16.000	640
2.4	6400	640	8000	200
2.5	5120	80	64.000	2560
Tn Cluster-KLH				
3.1	6400	1600	25.600	25.600
3.2	2560	3200	128.00025.600	
3.3	16.000	8000	128.00025.600	
3.4	640	12.800	5120	25.600
3.5	5120	12.800	25.600	3200
Tn-Cluster-BSA				
4.1	2560	12.800	2560	*
4.2	800	200	128.000400	
4.3	400	2560	6400	400
4.4	800	2560	12800	2560
4.5	1280	200	3200	3200

0.2 μg/well plated in ethanol.

*ND

Table 4. Tn-Cluster FACS Analysis; Serum Tested 11 Days Post 3rd Vaccination. FACS analysis using LSC cell line (Colon Cancer Cell line).

Group	IgG (% Gated)	IgM (% Gated)
Tn Cluster Pam		
1-1	93.95	16.59
1-2	19.00	66.15
1-3	54.45	40.51
1-4	46.99	39.98
1-5	3.07	32.83
Tn Cluster-Pam + QS-21		
2-1	12.00	76.78
2-2	2.48	36.76
2-3	20.27	46.41
2-4	10.64	55.29
2-5	3.37	38.95
Tn-Cluster-KLH		
3-1	96.36	66.72
3-2	93.12	45.50
3-3	97.55	32.96
3-4	94.72	49.54
3-5	83.93	64.33
Tn-Cluster-BSA		
4-1	80.65	41.43
4-2	90.07	31.68
4-3	42.86	54.03
4-4	95.70	63.76
4-5	92.14	51.89

Table 5. Results of Tn-trimer-Cys-KLH and Tn-trimer-Cys-BSA (MBS cross-linked) Conjugates

Conjugate	Amt of Carbohydrate & KLH used for Conjugation Carbo.	KLH	Final Conjugation Volume	Amt of Carbohydrate Recovered Carbohydrate	KLH	% Recovered Carbohydrate	KLH	µg of carbohydrate/100µl	µg of KLH/100µl
Tn-trimer-Cys-KLH	2.0 mg	5.0 mg	4.25 ml	141.174 µg	3612.5 µg	7%	72.25%	3.321	85
			2.5*				5.65	(3µg/mouse;300µl/vial¶)	
Tn-trimer-Cys-BSA	2.0	2.0	3.25	108.9	2762.5	5.445	100	3.35	85
			1*				10.89	(3µg/mouse;170µl/vial¶)	

*After concentration. ¶ Approximate amount.

A Total Synthesis of the Mucin Related F1α Antigen

The present invention provides derived mimics of surfaces of tumor tissues, based mainly on the mucin family of glycoproteins. Ragupathi, G., *et al., Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 125. (For a review of this area see Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, *24*, 231; Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.) Due to their high expression on epithelial cell surfaces and the high content of clustered O-linked carbohydrates, mucins constitute important targets for antitumor immunological studies. Mucins on epithelial tumors often carry aberrant α-O-linked carbohydrates. Finn, O.J., *et al., Immunol. Rev.* **1995**, *145*, 61; Saitoh, O. *et al., Cancer Res.* **1991**, *51*, 2854; Carlstedt, I.; Davies, J. R. *Biochem. Soc. Trans.* **1997**, *25*, 214. The identified F1α antigens **1'** and **2'** represent examples of aberrant carbohydrate epitopes found on mucins associated with gastric adenocarcinomas (Figure 22A). Yamashita, Y., *et al., J. Nat. Cancer Inst.* **1995**, *87*, 441; Yamashita, Y., *et al., Int. J. Cancer* **1994**, *58*, 349. Accordingly, the present invention provides a method of constructing the F1α epitope through synthesis. A previous synthesis of F1α is by Qui, D.; Koganty, R. R. *Tetrahedron Lett.* **1997**, *38*, 45. Other prior approaches to α-O-linked glycopeptides include Nakahara, Y., et al., in *Synthetic Oligasaccharides, Indispensable Probes for the Life Sciences* ACS Symp. *Ser. 560*, pp 249-266 (1994); Garg, H. G., *et al., Adv. Carb. Chem. Biochem.* **1994**, *50*, 277; Paulsen, H., *et al., J. Chem. Soc., Perkin Trans. 1*, **1997**, 281; Liebe, B.; Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 618; Elofsson, M., *et al., Tetrahedron* **1997**, *53*, 369; Meinjohanns, E., *et al., J. Chem. Soc., Perkin Trans. 1*, **1996**, 985; Wang, Z.-G., *et al., Carbohydr. Res.* **1996**, *295*, 25; Szabo, L., *et al., Carbohydr. Res.* **1995**, *274*, 11.

Tthe F1α structure could be constructed from the three principal building units I-III (Figure 22A). Such a general plan permits two alternative modes of implementation. (For a comprehensive overview of glycal assembly, see: Bilodeau, M. T.; Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1381. For applications toward the synthesis of carbohydrate tumor antigen based vaccines, see Sames, D., *et al., Nature* **1997**, *389*, 587; Park, T. K., *et al., J. Am. Chem. Soc.* **1996**, *118*, 11488; and Deshpande, P. P.;

Danishefsky, S. J. *Nature* **1997**, *387*, 164.) First, a GalNAc-serine/threonine construct might be assembled in the initial phase. This would be followed by the extension at the "non-reducing end" (II + III, then I). Alternatively, the entire glycodomain could be assembled first in a form of trisaccharide glycal (I + II). This step would be followed by coupling of the resultant trisaccharide donor to a serine or threonine amino acid residue (*cf.* II). Both strategies are disclosed herein.

The first synthetic approach commenced with preparation of monosaccharide donors **5a'/b'** and **6a'/b'** (Figure 22B). The protecting groups of galactal (*cf.* II) were carefully chosen to fulfill several requirements. They must be stable to reagents and conditions in the azidonitration protocol (*vide infra*). Also, the protecting functions must not undermine the coupling step leading to the glycosyl amino acid. After some initial experimentation, galactal **3'** became the starting material of choice. The azidonitration protocol (NaN_3, CAN CH_3CN, -20 °C) provided a 40% yield of 1:1 mixture of **4a'** and **4b'**. Lemieux, R. U.;Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244. Both anomers were hydrolyzed and then converted to a 1:5 mixture of trichloroacetimidates **5a'** and **5b'** in good yield (84%). Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 84. Alternatively, hydrolysis of nitrate **4'** followed by use of the DAST reagent (Rosenbrook, Jr. W., *et al.*, *Tetrahedron Lett.* **1985**, *26*, 3; Posner, G. H.; Haines, S. R. *Tetrahedron Lett.* **1985**, *26*, 5) yielded a 1:1 mixture of fluoride donors **6a'** and **6b'**. In both cases the α/β anomers were separable, thus allowing the subsequent investigation of their behavior in the coupling event. The best results obtained from the coupling of donors **5'-6'** to serine or threonine acceptors bearing the free side chain alcohol, with protected carboxy and amino moieties are summarized in Table 5a.

The trichloroacetimidate donor type **5'** provided excellent yields in coupling reactions with the serine derived alcohol **7'**. After optimization, donor **5b'** in the presence of TMSOTf in THF (entry 2, Table 5a) provided 86% yield of pure α-product **9'**. Interestingly, the donor **5a'** also provided α-glycoside **9'** exclusively. The coupling of donor **5b'** to threonine, though stereoselective, was low yielding. In this instance the

fluoride donors **6a'** and **6b'**, promoted by $Cp_2ZrCl_2/AgClO_4$ provided desired glycosyl threonine **10'** in excellent yield (82-87%) though with somewhat reduced selectivity (6:1, α:β). Ogawa, T. *Carbohydrate Res.* **1996**, *295*, 25. Thus, both sets of donors proved complementary to one another and glycosyl serine **9'** as well as glycosyl threonine **10'** were in hand in high yield and with excellent margins of stereoselectivity. It was found that the configurations at the anomeric centers of these donors had no practical effect on the stereochemical outcome of their coupling steps. This result differs from the finding with commonly used 2-*deoxy*-2-azido-tri-O-acetylgalactose-1-O-trichloroacetimidate. See Schmidt, R. R.; Kinzy, W., *id.* In that case each anomer yields a different ratio of α/β products (see below).

Table 5a.

x	Catalyst/promotor	R=H (**9'**) α:β (%)	R=CH_3 (**10'**) α:β (%)
-O(CNH)CCl_3(5b')	TMSOTf (0.1eq), CH_2Cl_2/Hex	7: 3 (100%)	7: 1 (33%)
-O(CNH)CCl_3(5b')	TMSOTf (0.5eq), THF	1: 0 (86%)	1 :0 (15%)
-O(CNH)CCl_3(5a')	TMSOTf (0.1eq), THF	1: 0 (66%)	____
-F (6a')	$Cp_2ZrCl_2/AgClO_4$ (2eq), CH_2Cl_2	2: 1 (89%)	6: 1 (87%)
-F(6b')	$Cp_2ZrCl_2/AgClO_4$ (2eq), CH_2Cl_2	2: 1 (91%)	6: 1 (82%)

The TIPS group at position 6 was quantitatively removed with TBAF and AcOH to give acceptors **11'** and **12'** (Figure 23). The final coupling to lactosamine donor **13'** was performed in the presence of $BF_3{\cdot}OEt_2$ in THF. The crude products from this apparently stereoselective coupling step were converted to compounds **14'** and **15'**, respectively with thiolacetic acid. Paulsen, H., *et al., Liebigs Ann.Chem.* **1994**, 381. These glycosyl amino acids represent suitable units for the glycopeptide assembly. In order to confirm their structure, we executed global deprotection. This was accomplished in five steps yielding free F1α antigen **1'** and **2'** in 70% and 73% yield, respectively (Figure 23). The glycosidic

linkages were not compromised under the conditions of the acidic and basic deprotection protocols.

A direct coupling Is provided of trisaccharide donors which are synthesized through glycal assembly (Bilodeau, M. T.; Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1381) using suitably protected serine or threonine amino acids. This logic was discussed earlier under the formalism I + II followed by coupling with III. The trisaccharide donors **23'-27'** were prepared as outlined in Figure 24. Readily available lactal **16'** (Kinzy, W.; Schmidt, R. R. *Carbohydrate Res.* **1987**, *164*, 265) was converted to the thio-donor **17'** via a sequence of the iodo-sulfonamidation and subsequent rearrangements with ethanethiol in the presence of LiHMDS. Park, T.K., *et al., J.Amer.Chem.Soc.*, **1996**, *118*, 11488. The MeOTf-promoted coupling to galactals **18'** and **19'** provided the trisaccharide glycals **20'** and **21'** in excellent yield and stereoselectivity. Reductive deprotection of the benzyl groups and the sulfonamide in **20'** and subsequent uniform acetylation of the crude product yielded glycal **22'**. The azidonitration of glycal **20'-22'** provided intermediate azidonitrates, which were converted to the corresponding donors **23'-27'**.

The results of couplings of these trisaccharide donors with suitable serine/threonine derived acceptors are summarized in Table 6. The protection pattern again had a profound effect on the reactivity and stereoselectivity of the coupling. Despite the seemingly large distance between the hydroxyl and other functional groups of the lactose domain from the anomeric center, these substituents strongly affects the stereochemical outcome. Qualitatively, uniform protection of functionality with electron donating groups (*cf.* benzyl) leads to a very reactive donor by stabilizing the presumed oxonium cation. By contrast, electron withdrawing protecting groups tend to deactivate the donor in the coupling step. Andrews, C. W., *et al., J. Org. Chem.* **1996**, *61*, 5280; Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656. Such deactivation may also confer upon a donor some stereochemical memory in terms of sensitivity of coupling to the original stereochemistry of the donor function at the anomeric center. As

shown in Table 6, per-O-benzyl-protected donor **23'** was highly reactive at -78°C providing product **28'** in 90% yield and high stereoselectivity (10:1, first entry, Table 6). A dramatic difference was seen upon changing the overall protection from per-O-benzyl to per-O-acetyl groups as demonstrated in the case of donor **24'**. The yield and stereoselectivity of the coupling step were diminished. Comparable results were obtained with donors **25'** and **26'**.

In the case of compounds **27'** and **28'**, where the galactosamine ring was conformationally restricted by engaging the 3- and 4-positions in the cyclic acetonide, an even more surprising finding was registered. Donor **27α'** with a per-O-benzyl protected lactosamine disaccharide afforded only the desired α-anomer **31'**. However, a mixture of trichloroacetimidates as well as the pure β anomer of **28'** yielded undesired β anomer **32'** exclusively. Thus, a modification of the protection pattern at a relatively distant site on the second and third carbohydrate units (from the ring containing the donor function) exerted a profound reversing effect on the stereoselectivity of glycosidation. Conformational limitations imposed on a ring within the donor ensemble by cyclic protecting groups can influence donor reactivity, as judged by rates of hydrolysis. Wilson, B. G.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 317; Fraser-Reid, B., *et al.*, *J.Am.Chem.Soc*,. **1991**, *113*, 1434. Protecting groups, *via* their electronic, steric and conformational influences, coupled with solvation effects, can strongly modulate the characteristics of glycosyl donors. Thus, longer range effects cannot be accurately predicted in advance in the glycosidation of serine and threonine side chain hydroxyls.

Table 6.

R_1	R_2	R_3	X	R_4	Catalyst/Promotor	α:β (%)
Bn	Bn	$PhSO_2HN$	O(CNH)CCl_3 (**23**´α)	Me	TMSOTf (0.5eq), THF	10:1 (90%) **29**´
Ac	Ac	AcHN	O(CNH)CCl_3 (**24**´α/β 3:1)	Bn	TMSOTf (1.0eq), THF	2:1 (22%) **30**´
Ac	Ac	AcHN	Br (**25**´α)	Bn	$AgClO_4$ (1.5eq), CH_2Cl_2	3.5:1 (56%) **30**´
Ac	Ac	AcHN	SPh (**26**´β)	Bn	NIS/TfOH, CH_2Cl_2	2:1 (40%) **30**´
Me_2C	Bn	AcHN	O(CNH)CCl_3 (**27**´α)	Bn	TMSOTf (0.3eq), THF	1:0 (50%) **31**´
Me_2C	Ac	N_3	O(CNH)CCl_3 (**28**´α/β 1:1)	Bn	BF_3Et_2O (0.5eq), THF	0:1 (67%) **32**´
Me_2C	Ac	N_3	O(CNH)CCl_3 (**28**´β)	Bn	BF_3Et_2O (1.5eq), THF	0:1 (35%) **32**´

Accordingly, the present invention demonstrates unexpected advantages for the cassette approach wherein prebuilt stereospecifically synthesized α-O-linked serine or threonine glycosides (*e.g.*, **9'** and **10'**) are employed to complete the saccharide assembly.

Probing Cell Surface Architecture through Total Synthesis: Immunological Consequences of a Human Blood Group Determinant in a Clustered Mucin-like Context

Blood group antigens were initially defined as carbohydrate structures on the surface of red blood cells. However, many blood group antigens such as those of the ABH and Lewis systems are not solely erythrocyte-associated, but are more broadly distributed as the terminal carbohydrate moieties on glycoproteins and glycolipids in many epithelia and their secretions. Greenwell, P. *Glycoconjugate J.*, **1997**, *14*, 159-173. Protein-bound blood group determinants are often encountered in a mucin-like context in which they are *O*-linked *via* an *N*-acetylgalactosamine residue to hydroxyl groups of serine or threonine residues. Müller, S., *et al. J. Biol. Chem.*, **1997**, *272*, 24780-24793. The precise functions of the blood groups have not been defined, but the structural variability of this system may be preserved as part of a defense strategy against invading microorganisms bearing foreign cell-surface antigens, also some Lewis epitopes are involved in cell adhesions mediated by selectins. Varki, A. *Proc.*

Natl. Acad. Sci. USA, **1994**, *91*, 7390-7397. Altered expressions of certain blood-group antigens on tumor cells can serve as tumor markers in a variety of carcinomas. Lloyd, K. O. *Am. J. Clin. Pathol.*, **1987**, *87*, 129-139. One such example is the enhanced presentation of the Lewis^y (Le^y) histo-blood determinant [Fuca1-2Galb1-4(Fuca1-3)GlcNAc] in mucin or glycolipid form on many human tumor cells, including those found in colon, lung, breast, and ovarian cancers. Yin, B. W. T., *et al. Int. J. Cancer*, **1996**, *65*, 406-412. In mucins, this blood group determinant is carried in clustered motifs on adjacent or closely spaced serine and threonine residues. Müller, S., *supra*. The isolation of homogeneous mucin segments, containing such clustered blood group determinants, from natural sources, would be immensely complicated due to microheterogeneity, in addition to the requirement of achieving proteolysis of glycoproteins at fixed points. The availability of realistic and homogeneous mucin fragments would be of considerable advantage in facilitating biological and structural studies. The complexity of the issues to be overcome in pursuit of a fully synthetic homogeneous blood group determinant in a clustered setting presented a clear challenge to the science of chemical synthesis. The present invention provides a solution to the problem in the context of a total synthesis of Le^y-containing glycopeptides in mucin form.

In designing the Le^y mucin mimic, the following features were incorporated: (i) presentation of the full Le^y tetrasaccharide, (ii) incorporation of an intervening carbohydrate spacer group so that the structure and immunological integrity of the determinants are not altered or dwarfed by direct contact with the protein-like domain, (iii) an option for clustering *via* suitable peptide couplings, and (iv) provisions for installation of a flanking sequence linked through the carboxy terminus culminating in the immunostimulating Pam_3Cys moiety. Bessler, W. G., *et al. J. Immunol.*, **1985**, *135*, 1900-1905; Toyokuni, T., Hakomori, S.-I., Singhal, A. K. *Bioorg. Med. Chem.*, **1994**, *2*, 1119-1132. In this way it was possible to circumvent the need for conjugation of the complex construct to a carrier protein such as KLH to induce immunogenicity. Thus far, such protein-carbohydrate conjugations are achieved only in limited yields. The wide range of protecting groups required for such a synthesis proved to present a major strategic problem now overcome by the present inventors.

The synthetic plan provided herein drew from two methodological advances developed by the present inventors. The first is the strategy of glycal assembly for the rapid buildup of oligosaccharides. Danishefsky, S. J., Bilodeau, M. T. *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 1380-1419. The second is the newly introduced "cassette" method for solving the stereochemical problems associated with constructing α-serine (threonine) *O*-linked oligosaccharides. Kuduk, S. D., *et al. J. Am. Chem. Soc.*, **1998**, *120*, 12474-12485; Schwarz, B., *et al. J. Am. Chem. Soc.*, in press. In the cassette strategy, an *N*-acetylgalactosamine synthon is made stereospecifically α-*O*-linked to a serine (or threonine) residue with a differentiable acceptor site on the GalNAc. This construct serves as a general insert (cassette) that is joined to a target saccharide bearing a glycosyl donor function at its reducing end. In this way, the need is avoided for direct coupling of the serine side-chain hydroxyl group to a fully elaborated, complex saccharide donor. The classical method, as opposed to the cassette approach, tends to provide complex stereochemical mixtures. For the case at hand, in the interest of synthetic conciseness, cassette **2A** containing undifferentiated acceptor sites at C3 and C4 was used. In fact, owing to the equatorial nature of the C3 hydroxyl, glycosidation occurred only at this position (*vide infra*).

The pentasaccharide glycal (Danishefsky, S. J., *et al., J. Am. Chem. Soc.*, **1995**, *117*, 5701-5711) was prepared via the glycal assembly methodology as shown, and converted to the thioethyl donor **1A** in accord with previously described chemistry. Seeberger, P. H., *et al., J. Am. Chem. Soc.*, **1997**, *119*, 10064-10072. Thus, a stereospecific cassette route to the complex *O*-linked oligosaccharides was implemented. Reaction of donor **1A** with cassette acceptor **2A** (Kuduk, *supra*) under NIS/TfOH conditions (Konradsson, P., *et al., Tetrahedron Lett.*, **1990**, *31*, 4313-4316; Veeneman, G. H., *et al., Tetrahedron Lett.*, **1990**, *31*, 1331-1334) afforded the coupled product bearing the required serine α-*O*-linked to a complex carbohydrate domain. Functional group management, as shown, led to acid **3A**. The mucin construction necessitated peptide couplings of highly complex glycosylamino acids. HOAt/HAtU methodology (Carpino, L. A. *J. Am. Chem. Soc.*, **1993**, *115*, 4397-4398) allowed for efficient assembly of the linear heptapeptide mucin model precursor **4A**. Following

removal of the Fmoc-protecting group, the free amine was capped by acetylation. Hydrogenolytic cleavage of the benzyl ester exposed the fully protected C-terminal carboxyl. In the culminating global deprotection step, treatment with hydrazine hydrate in methanol smoothly cleaved the acetate and benzoate esters to afford the fully deprotected glycopeptide. The success of the hydrazinolysis step was crucial since the benzoate protecting groups on the three galactose spacers (see asterisks) insulating the blood group determinant from the serine residues had resisted typical deprotection conditions (pH 10 aq. NaOH/MeOH, LiOH, LiOOH, and cat. NaOMe/MeOH). Finally, the lipid amine **5A** was coupled to the acid terminus of the heptapeptide under the conditions shown to afford the synthetic antigenic construct **6A**.

Three additional pentasaccharide-based constructs lacking the internal galactose (see **7A** to **9A**) were prepared through a conceptually related route; a trisubstituted lipopeptide (**7A**) retaining the α-GalNAc linkage of **6A**, a similar construct with a β-linked GalNAc (**8A**), and a singly Le^y-substituted lipopeptide (**9A**) (Figure 29). In this route, without the cassette logic, the glycopeptide synthesis was nonstereospecific. Immunological evaluations were conducted in the series **7A-9A** where comparisons were possible.

Immunological Results.

The reactivities of Le^y-containing lipoglycopeptide constructs (**6A-9A**), as well as the control compound, Le^y-ceramide (**10A**) (Kudryashov, V., *et al., Cancer Immunol. Immunother.*, **1998**, 45, 281-286), to anti-Le^y antibody 3S193 (Kitamura, K. *et al. Proc. Nat. Acad. Sci. (Wash.),* **1994**, *91*, 12957-12961) were determined by ELISA assay (Figure 30). This antibody had been elicited by tumor cells that presumably display the cell surface mucin motif. Of the synthesized constructs, the α-O-linked hexasaccharide **6A** and the β-O-linked Le^y-containing glycopeptide **8A** were the most reactive and were comparable to the Le^y-ceramide control, **10A**. The α-O-linked monomer and trimeric constructs (**7A** and **9A**, respectively) showed similar reactivity to one another, but were significantly less well bound than the control. These results suggest that the constructs having a β-linkage for the

attachment of the terminal pentasaccharide most closely resembles the tumor-expressed, cell-surface Le^y against which the antibody 3S193 was elicited.

Mice were immunized with the Le^y-pentasaccharide constructs without adjuvant and the antisera were tested against Le^y-ceramide, Le^y-mucin, and Le^y-expressing tumor cells to examine the effects of antigen structure on immunogenicity and the tumor cell reactivity of the antibody response. Clustering of the glycodomain was found to be crucial for antibody production to natural substrates. The α- and β-*O*-linked trimeric structures (**7A** and **8A**) are highly immunogenic with levels of antibody response to Le^y-ceramide and Le^y-mucin comparable to Le^y-KLH (Kudryashov, V., *supra*), whereas the immunological response of the monomeric construct **9A** to the same targets was poor. (See Figure 31) The same trend was observed in FACS analysis of cell surface reactivity; antisera produced against the clustered motifs each bound to approximately 74% of the Le^y-expressing tumor cells whereas the monomeric-Le^y-derived antisera bound approximately 58% of the cells. (Table 7) In addition, the natural glycosidic linkage to the amino acid that is found in mucin glycoproteins is not critical for antibody production to Le^y-bearing glycolipids and mucin. In fact, the unnatural GalNAc-β-*O*-Ser-linked construct is equally immunogenic to the α-*O*-Ser form. It is possible that GalNAc-β1- closely resembles the Gal-β1- that would be found in natural glycan chains. The antibody response to the lipoglycopeptide constructs was primarily IgM, whereas Le^y-KLH produced IgG as well as IgM antibodies. Kudryashov, V., *supra*. It appears that the Pam_3Cys immunomodulating unit stimulated only B cells in the study.

The possibility of using completely synthetic carbohydrate-based constructs opens up new opportunities for the vaccine therapy of cancer. Most cancer vaccines used to date have employed oligosaccharides artificially linked to natural proteins, such as KLH or tetanus toxoid, together with immunoadjuvants (e.g., alum, Detox (MacLean, G. D., *et al., J. Immunother.*, **1996**, *19*, 59-68,), or QS21 (Livingston, P. O., et *al., Vaccine,* **1994**, *12*, 1275-1280), a saponin derivative). The use of fully synthetic constructs simplifies manufacturing and regulatory processes. This study also reveals the ability of a clustered oligosaccharide structure to stimulate an antibody response that is superior in terms of its reactivity with

natural antigens and cells. A similar effect is seen for a clustered sialyl-Tn construct, thus illustrating the generality of the procedure. Ragupathi, G., *et al.*, *Cancer Immunol. Immunother.*, in press. It has been shown previously that some antibodies, *e.g.*, B72.3 or MLS 128, that were raised to tumor cells detect epitopes encompassing clustered motifs (Zhang, S., *et al.*, *Can. Res.*, **1995**, *55*, 3364-3368; Nakada, H., *et al.*, *Proc. Nat'l Acad. Sci. USA.*, **1993**, *90*, 2495-2499), but this is the first demonstration of the inverse, *i.e.*, that immunization with synthetic antigens having clustered structures mimics immunization with cells or natural antigens.

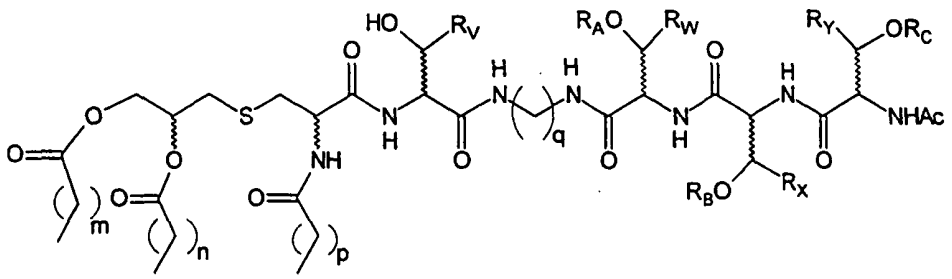
Table 7. Reactivity of mice sera with Le^y-expressing OVCAR-3 ovarian cancer cells as analyzed by fluorescence-activated cell sorting (FACS).

Mice	Immunogen	percent positive cells[a]		
Group A	(α-Le^y-penta)$_3$-PamCys (**7A**)	73.5 ± 4.5		p = 0.08
Group B	(β-Le^y-penta)$_3$-PamCys (**8A**)	73.7 ± 2.7	p = 0.08	
Group C	(α-Le^y-penta)$_1$-PamCys (**9A**)	57.4 ± 10.6		

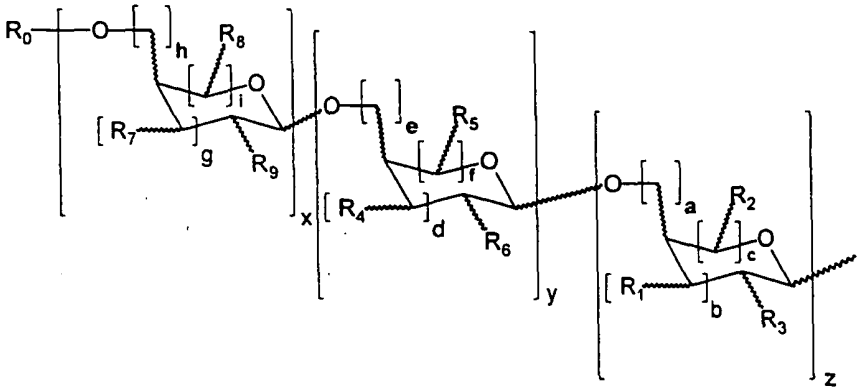
[a] Average and s.d. of 5 mice per group. Fluorescence given by pre-immunized sera was gated at 8-10% of positive cells. Mouse sera was diluted 1:20 for these assays. No reactivity was observed with the Le^y-negative melanoma cell line SK-MEL-28.

What Is Claimed Is:

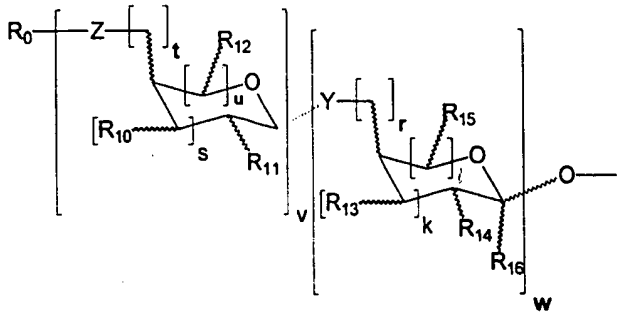
1. A glycoconjugate having the structure:



wherein *m*, *n* and *p* are integers between about 8 and about 20; wherein *q* is an integer between about 1 and about 8; wherein R_V, R_W, R_X and R_Y are independently hydrogen, optionally substituted linear or branched chain lower alkyl or optionally substituted phenyl; wherein R_A, R_B and R_C are independently a carbohydrate domain having the structure:



wherein *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *x*, *y* and *z* are independently 0, 1, 2 or 3; wherein R_0 is hydrogen, linear or branched chain lower alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyl-oxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein *k*, *l*, *r*, *s*, *t*, *u*, *v* and *w* are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, optionally substituted linear or branched chain lower alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently hydrogen, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group.

2. The glycoconjugate of claim 1 wherein R_V, R_W, R_X and R_Y are methyl.

3. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently monosaccharides or disaccharides.

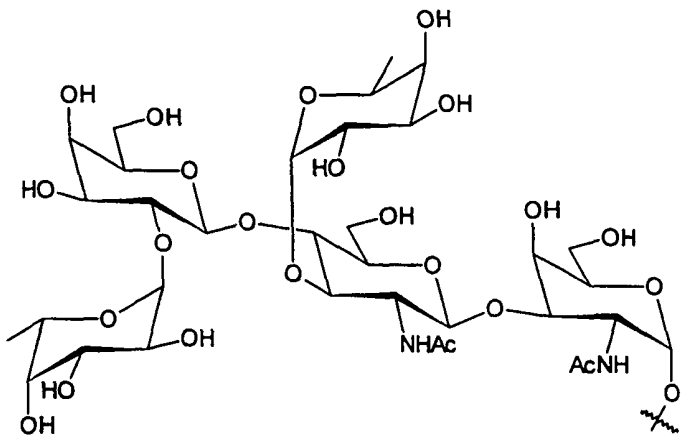
4. The glycoconjugate of claim 3 wherein *y* and *z* are 0; wherein *x* is 1; and wherein R_3 is NHAc.

5. The glycoconjugate of claim 1 wherein *h* is 0; wherein *g* and *i* are 1; wherein R_7 is OH; wherein R_0 is hydrogen; and wherein R_8 is hydroxymethyl.

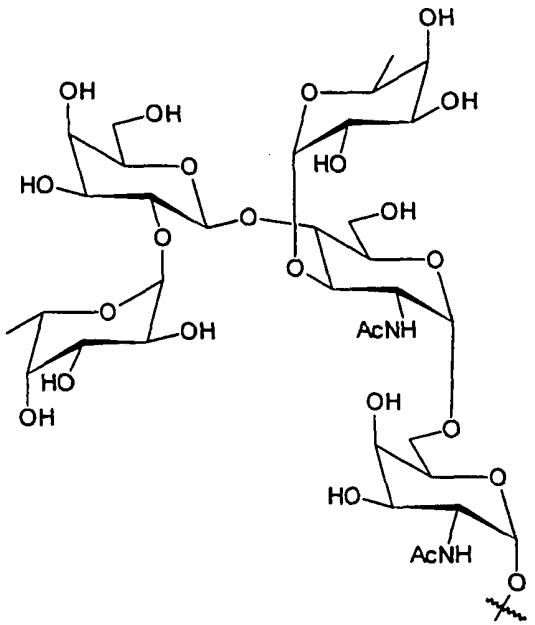
6. The glycoconjugate of claim 1 wherein *m*, *n* and *p* are 14; and wherein q is 3.

7. The glycoconjugate of claim 1 wherein each amino acyl residue therein has an L-configuration.

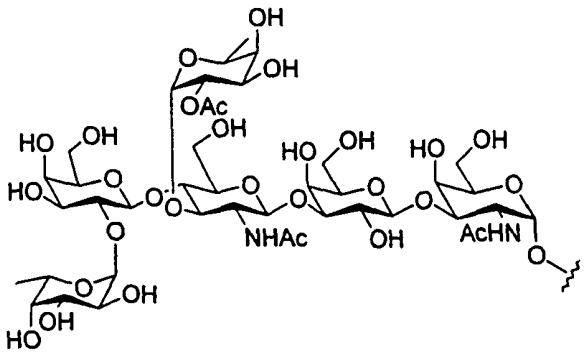
8. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



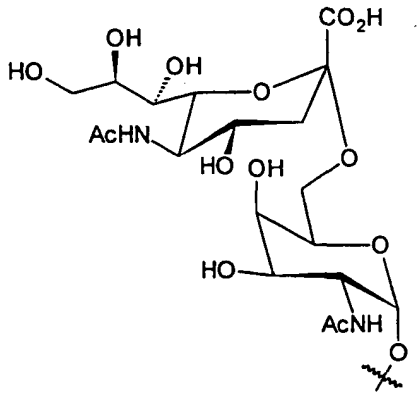
9. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



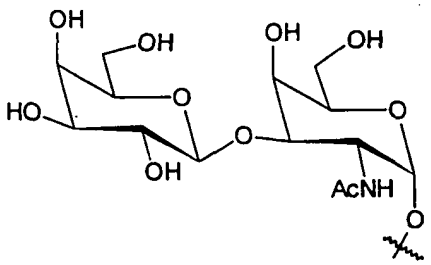
10. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



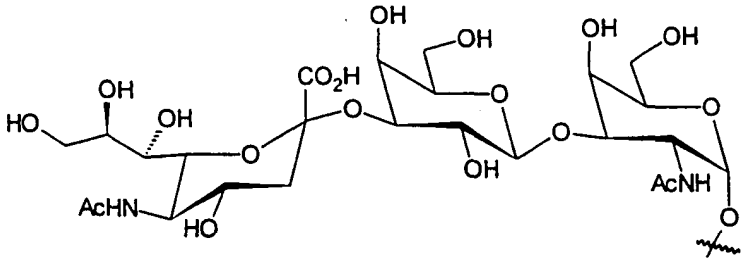
11. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



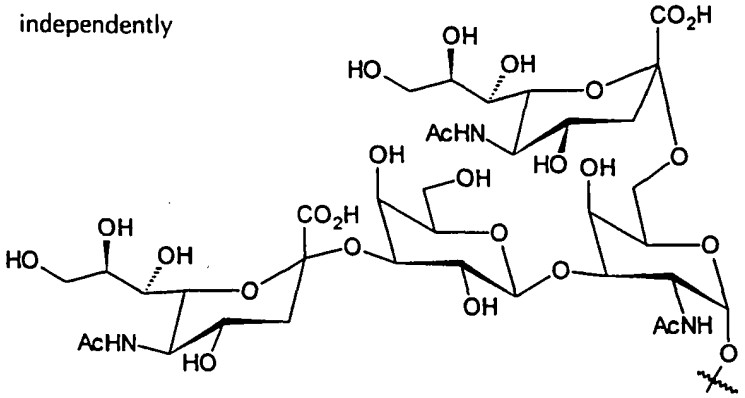
12. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



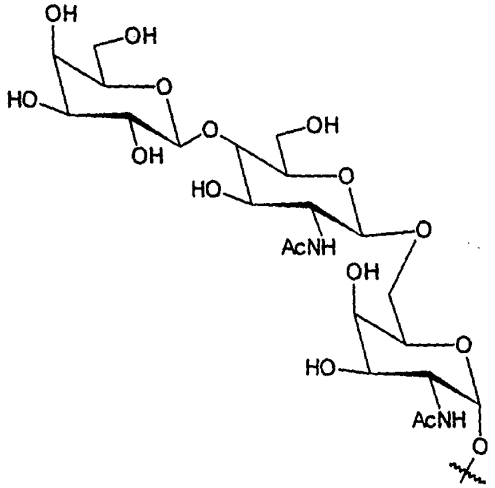
13. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



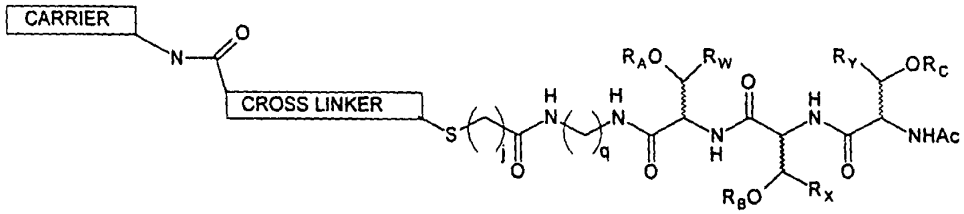
14. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



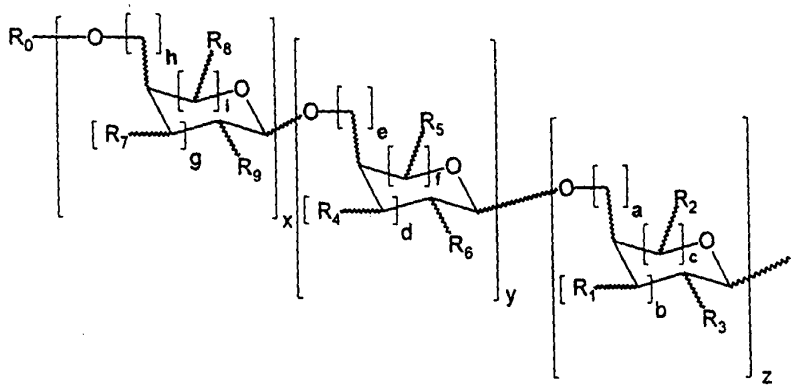
15. The glycoconjugate of claim 1 wherein the carbohydrate domains are independently



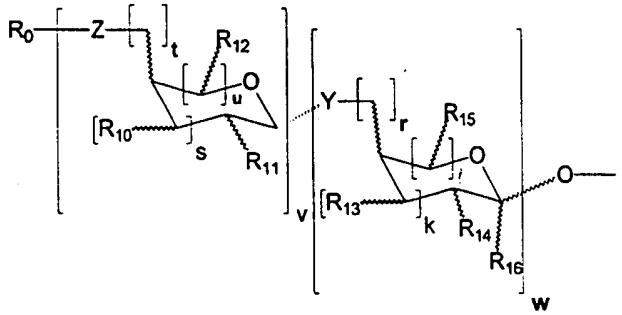
16. A glycoconjugate having the structure:



wherein the carrier is a protein; wherein the cross linker is a moiety derived from a cross linking reagent capable of conjugating a surface amine of the carrier and a thiol; wherein *m*, *n* and *p* are integers between about 8 and about 20; wherein *j* and *q* are independently integers between about 1 and about 8; wherein R_v, R_w, R_x and R_y are independently hydrogen, optionally substituted linear or branched chain lower alkyl or optionally substituted phenyl; wherein R_A, R_B and R_C are independently a carbohydrate domain having the structure:

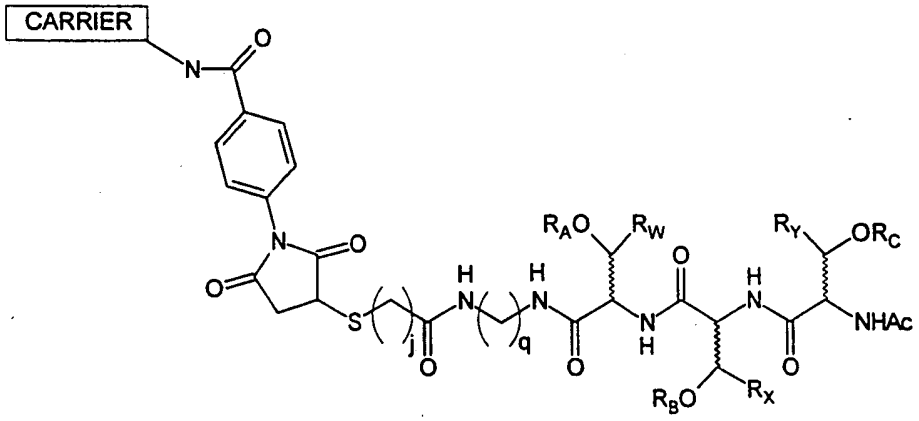


wherein a, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *x*, *y* and *z* are independently 0, 1, 2 or 3; wherein R_0 is hydrogen, linear or branched chain lower alkyl, acyl, arylalkyl or aryl group; wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each independently hydrogen, OH, OR^i, NH_2, $NHCOR^i$, F, CH_2OH, CH_2OR^i, an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyl-oxyalkyl, arylalkyl or aryl group; wherein R^i is hydrogen, CHO, $COOR^{ii}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein *k*, *l*, *r*, *s*, *t*, *u*, *v* and *w* are each independently 0, 1 or 2; wherein R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently hydrogen, OH, OR^{iii}, NH_2, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or an optionally substituted linear or branched chain lower alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, optionally substituted linear or branched chain lower alkyl or aryl group; wherein R^{iii} is hydrogen, CHO, $COOR^{iv}$, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group; and wherein R^{ii} and R^{iv} are each independently hydrogen, or an optionally substituted linear or branched chain lower alkyl, arylalkyl or aryl group.

17. The glycoconjugate of claim 16 having the structure:



18. The glycoconjugate of claim 16 wherein R_v, R_w, R_x and R_y are methyl.

19. The glycoconjugate of claim 16 wherein the carbohydrate domains are monosaccharides or disaccharides.

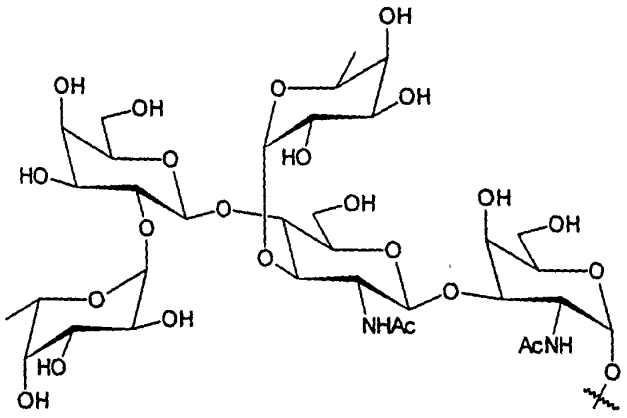
20. The glycoconjugate of claim 19 wherein *y* and *z* are 0; wherein x is 1; and wherein R_3 is NHAc.

21. The glycoconjugate of claim 16 wherein *h* is 0; wherein *g* and *i* are 1; wherein R_7 is OH; wherein R_6 is hydrogen; wherein *m*, *n* and *p* are 14; and wherein q is 3; and wherein R_8 is hydroxymethyl.

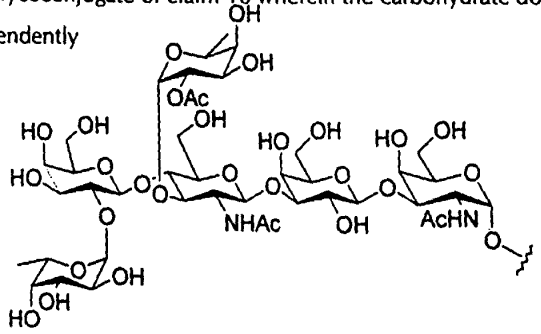
22. The glycoconjugate of claim 16 wherein the protein is BSA or KLH

23. The glycoconjugate of claim 16 wherein each amino acyl residue therein has an L-configuration.

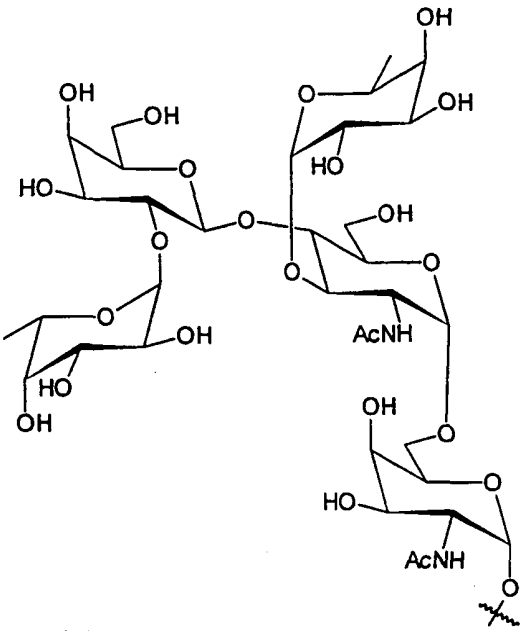
24. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



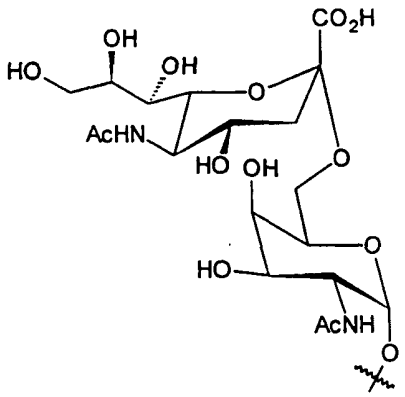
25. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



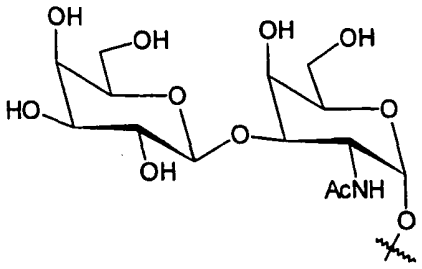
26. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



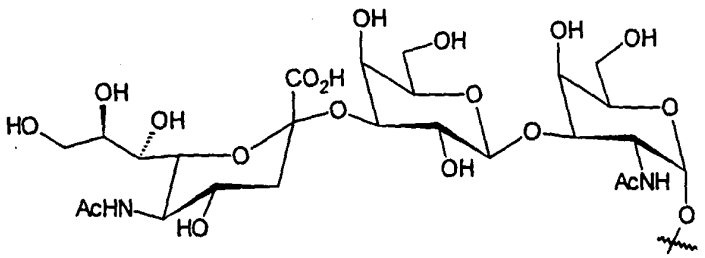
27. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



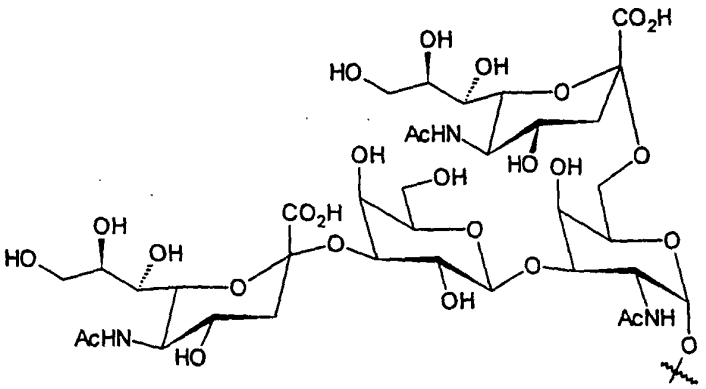
28. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



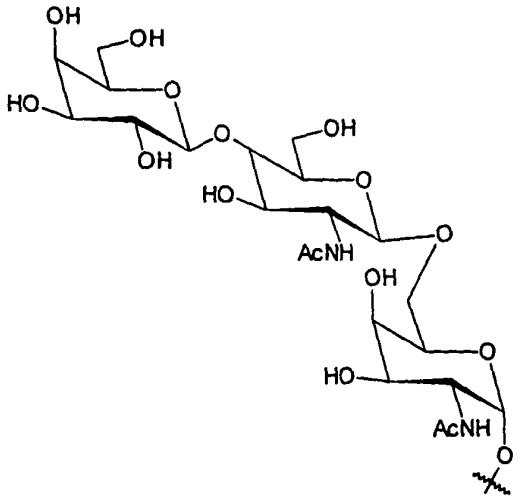
29. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



30. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



31. The glycoconjugate of claim 16 wherein the carbohydrate domains are independently



32. A pharmaceutical composition for treating cancer comprising a glycoconjugate of claim 1 or 16 and a pharmaceutically suitable carrier.

33. A method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of a glycoconjugate of claim 1 or 16 and a pharmaceutically suitable carrier.

34. The method of claim 33 wherein the cancer is a solid tumor.

35. The method of claim 33 wherein the cancer is an epithelial cancer.

36. A method of inducing antibodies in a human subject, wherein the antibodies are capable of specifically binding with human tumor cells, which comprises administering to the subject an amount of the glycoconjugate of claim 1 or 16 effective to induce the antibodies.

37. The method of claim 36 wherein the carrier protein is bovine serum albumin, polylysine or KLH.

38. The method of claim 36 which further comprises co-administering an immunological adjuvant.

39. The method of claim 38 wherein the adjuvant is bacteria or liposomes.

40. The method of claim 38 wherein the adjuvant is *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

41. The method of claim 36 wherein the antibodies induced are selected from the group consisting of Tn, ST_N, (2,3)ST, glycophorine, 3-Le^y, 6-Le^y, T(TF) and T antibodies.

42. The method of claim 36 wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.

43. A method of preventing recurrence of epithelial cancer in a subject which comprises vaccinating the subject with the glycoconjugate of claim 1 or 16 which amount is effective to induce antibodies.

44. The method of claim 43 wherein the carrier protein is bovine serum albumin, polylysine or KLH.

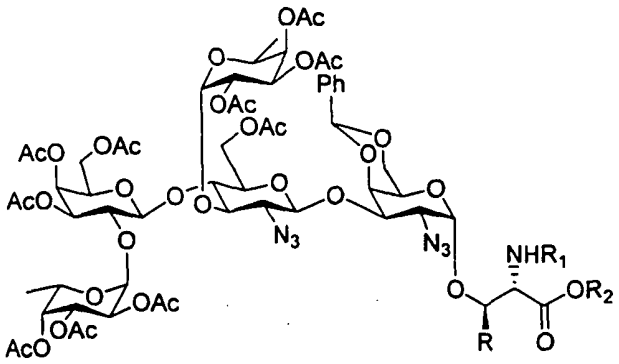
45. The method of claim 43 which further comprises co-administering an immunological adjuvant.

46. The method of claim 45 wherein the adjuvant is bacteria or liposomes.

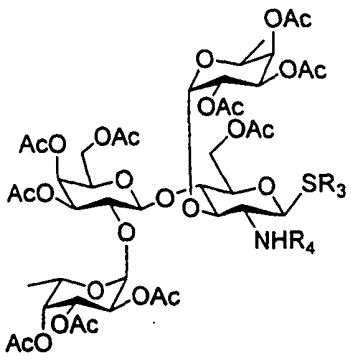
47. The method of claim 45 wherein the adjuvant is *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

48. The method of claim 43 wherein the antibodies induced are selected from the group consisting of Tn, ST_N, (2,3)ST, glycophorine, 3-Le^y, 6-Le^y, T(TF) and T antibodies.

49. A method of preparing a protected O-linked Le^y glycoconjugate having the structure:

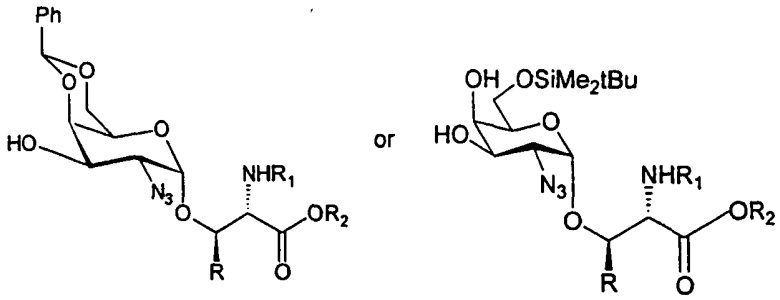


wherein R is hydrogen, linear or branched chain lower alkyl, or optionally substituted aryl; R_1 is t-butyloxycarbonyl, fluorenylmethyleneoxycarbonyl, linear or branched chain lower alkyl or acyl, optionally substituted benzyl or aryl; R_2 is a linear or branched chain lower alkyl, or optionally substituted benzyl or aryl; and R_4 is hydrogen, linear or branched chain lower alkyl or acyl, optionally substituted aryl or benzyl, or optionally substituted aryl sulfonyl; which comprises coupling a tetrasaccharide sulfide having the structure:



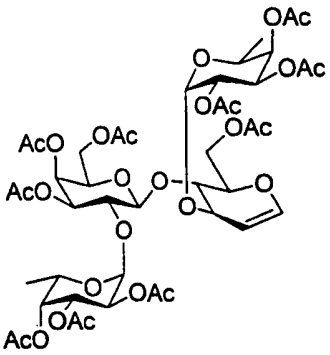
wherein R_3 is linear or branched chain lower alkyl or aryl; with an O-linked

glycosyl amino acyl component having the structure:



under suitable conditions to form the protected O-linked Le^y glycoconjugate.

50. The method of claim 49 wherein the tetrasaccharide sulfide is prepared by (a) halosulfonamidating a tetrasaccharide glycal having the structure:

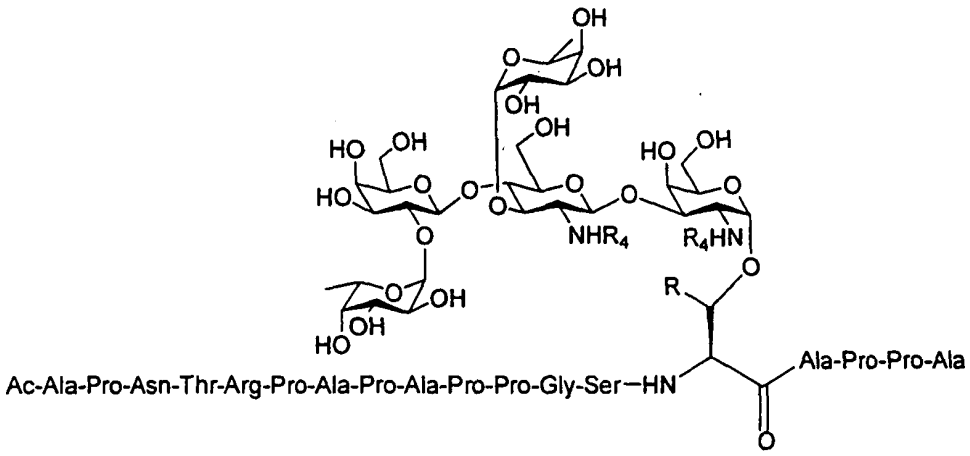


under suitable conditions to form a tetrasaccharide halosulfonamidate; and (b) treating the halosulfonamidate with a mercaptan and a suitable base to form the tetrasaccharide sulfide.

51. The method of claim 50 wherein the mercaptan is a linear or branched chain lower alkyl or an aryl; and the base is sodium hydride, lithium hydride, potassium hydride, lithium diethylamide, lithium diisopropylamide, sodium amide, or lithium hexamethyldisilazide.

52. An O-linked glycoconjugate prepared in accord with claim 49.

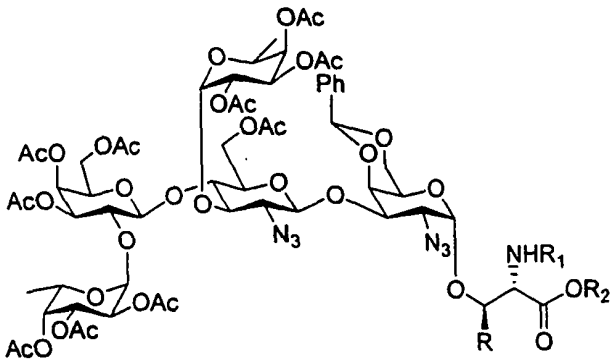
53. An O-linked glycopeptide having the structure:



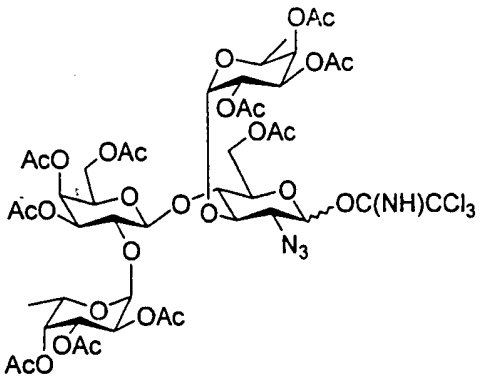
wherein R_4 is a linear or branched chain lower acyl; and wherein R is hydrogen or a linear or branched chain lower alkyl or aryl.

54. The O-linked glycopeptide of claim 53 wherein R_4 is acetyl.

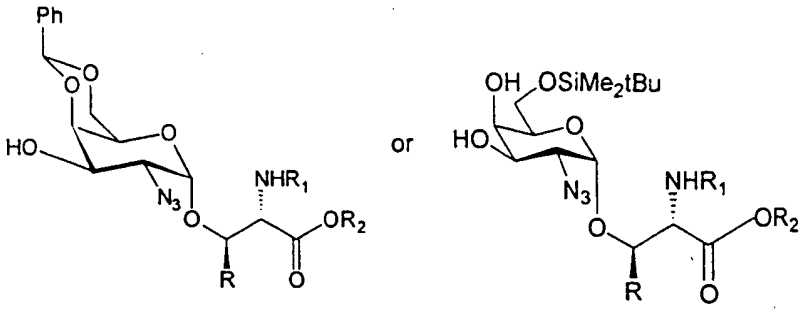
55. A method of preparing a protected O-linked Le^y glycoconjugate having the structure:



wherein R is hydrogen, linear or branched chain lower alkyl, or optionally substituted aryl; R_1 is t-butyloxycarbonyl, fluorenylmethyleneoxycarbonyl, linear or branched chain lower alkyl or acyl, optionally substituted benzyl or aryl; and R_2 is a linear or branched chain lower alkyl, or optionally substituted benzyl or aryl; which comprises coupling a tetrasaccharide azidoimidate having the structure:

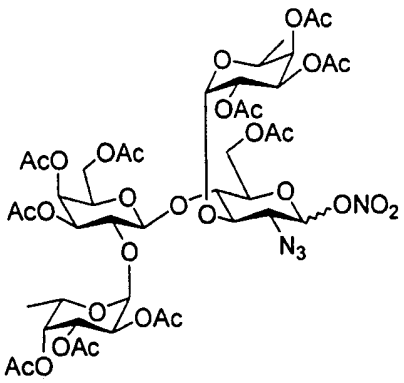


with an O-linked glycosyl amino acyl component having the structure:



under suitable conditions to form the protected O-linked Le^y glycoconjugate.

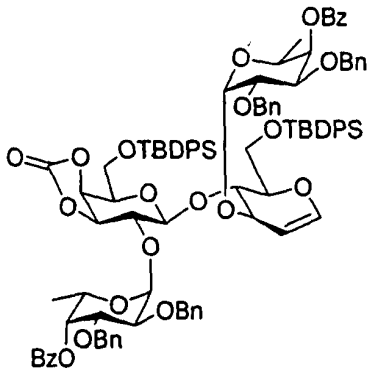
56. The method of claim 55 wherein the tetrasaccharide azidoimidate is prepared by (a) treating tetrasaccharide azidonitrate having the structure:



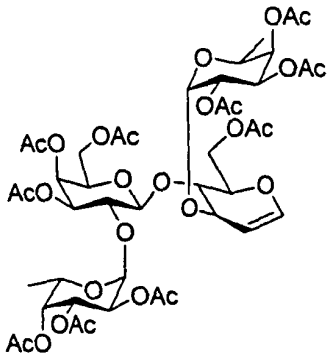
under suitable conditions to form an azido alcohol; and (b) reacting the azido alcohol with an imidoacylating reagent under suitable conditions to form the azidoimidate.

57. The method of claim 56 wherein the tetrasaccharide azido nitrate is prepared by (a)

converting a tetrasaccharide glycal having the structure:



under suitable conditions to a peracetylated tetrasaccharide glycal having the structure:



and (b) azidonitrating the glycal formed in step (a) under suitable conditions to form the tetrasaccharide azido nitrate.

57. The method of claim 56 wherein step (b) is effected using cerium ammonium nitrate in the presence of an azide salt selected from the group consisting of sodium azide, lithium azide, potassium azide, tetramethylammonium azide and tetraethylammonium azide.

58. An O-linked glycoconjugate prepared in accord with claim 55.

FIG. 1

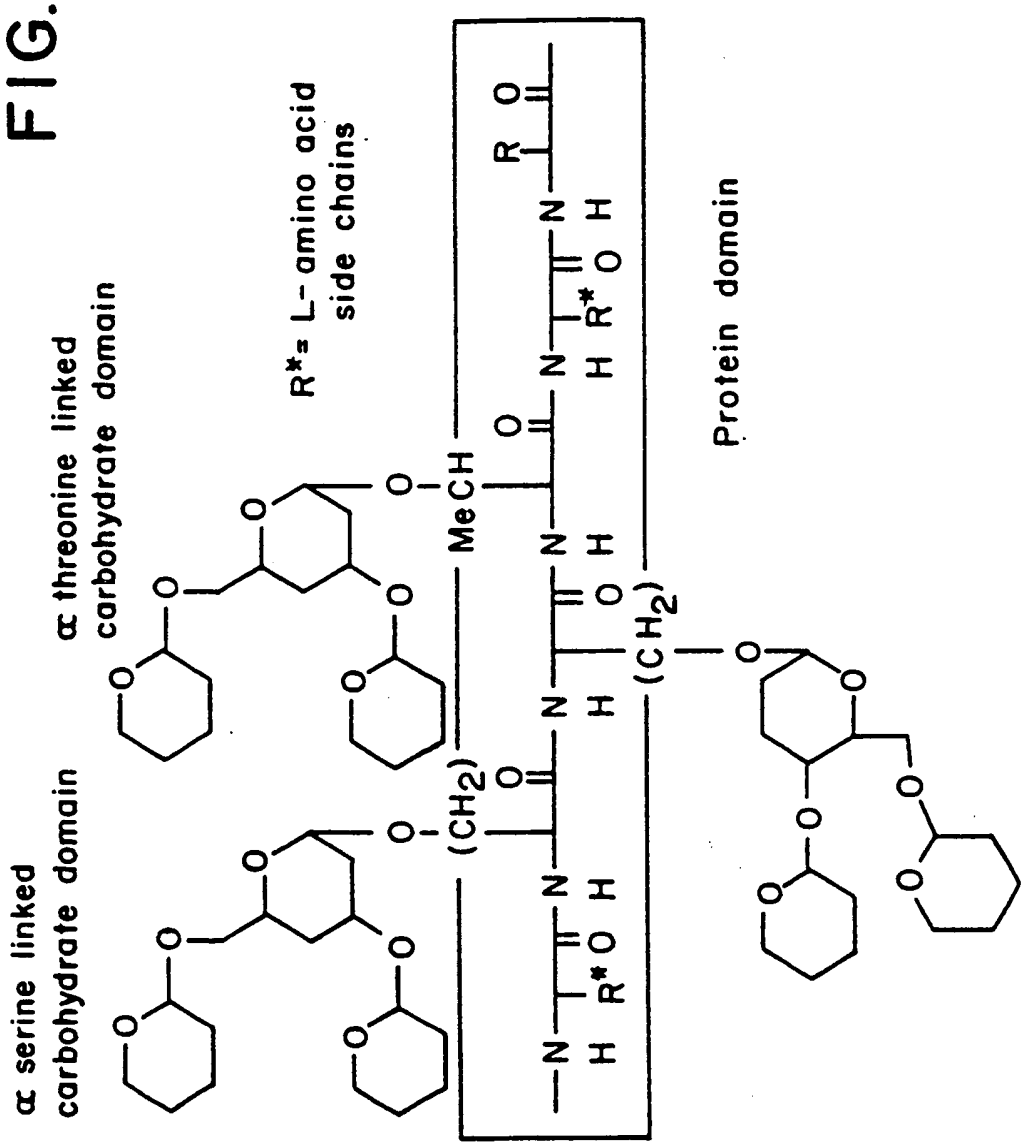

α serine linked carbohydrate domain
α threonine linked carbohydrate domain
R*= L-amino acid side chains
Protein domain
MeCH
(CH2)
(CH2)

FIG. 2A

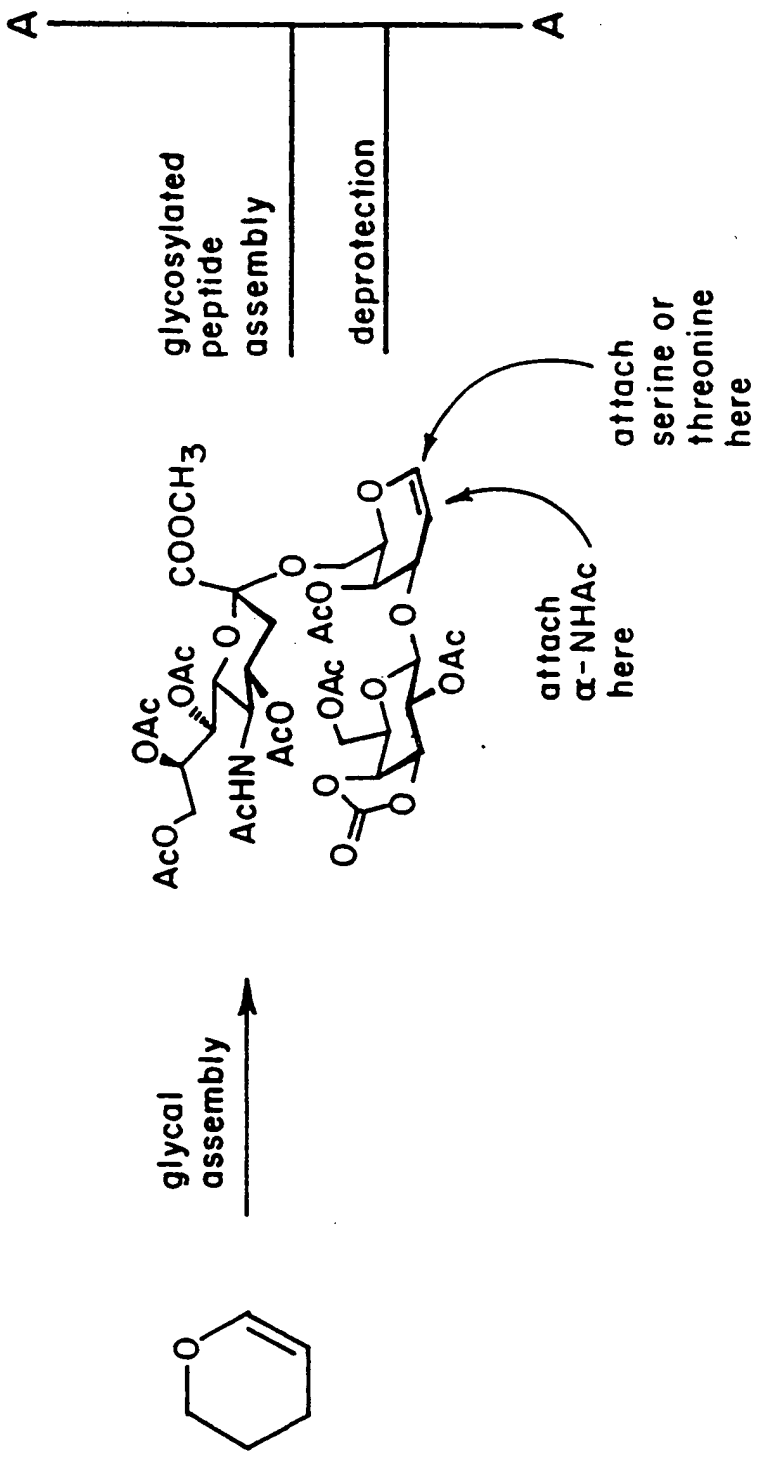

A
A
glycosylated peptide assembly
deprotection
attach serine or threonine here
attach α-NHAc here
glycal assembly
COOCH3
OAc
AcO
AcHN
AcO
OAc
AcO
OAc
OAc
O

FIG. 2B

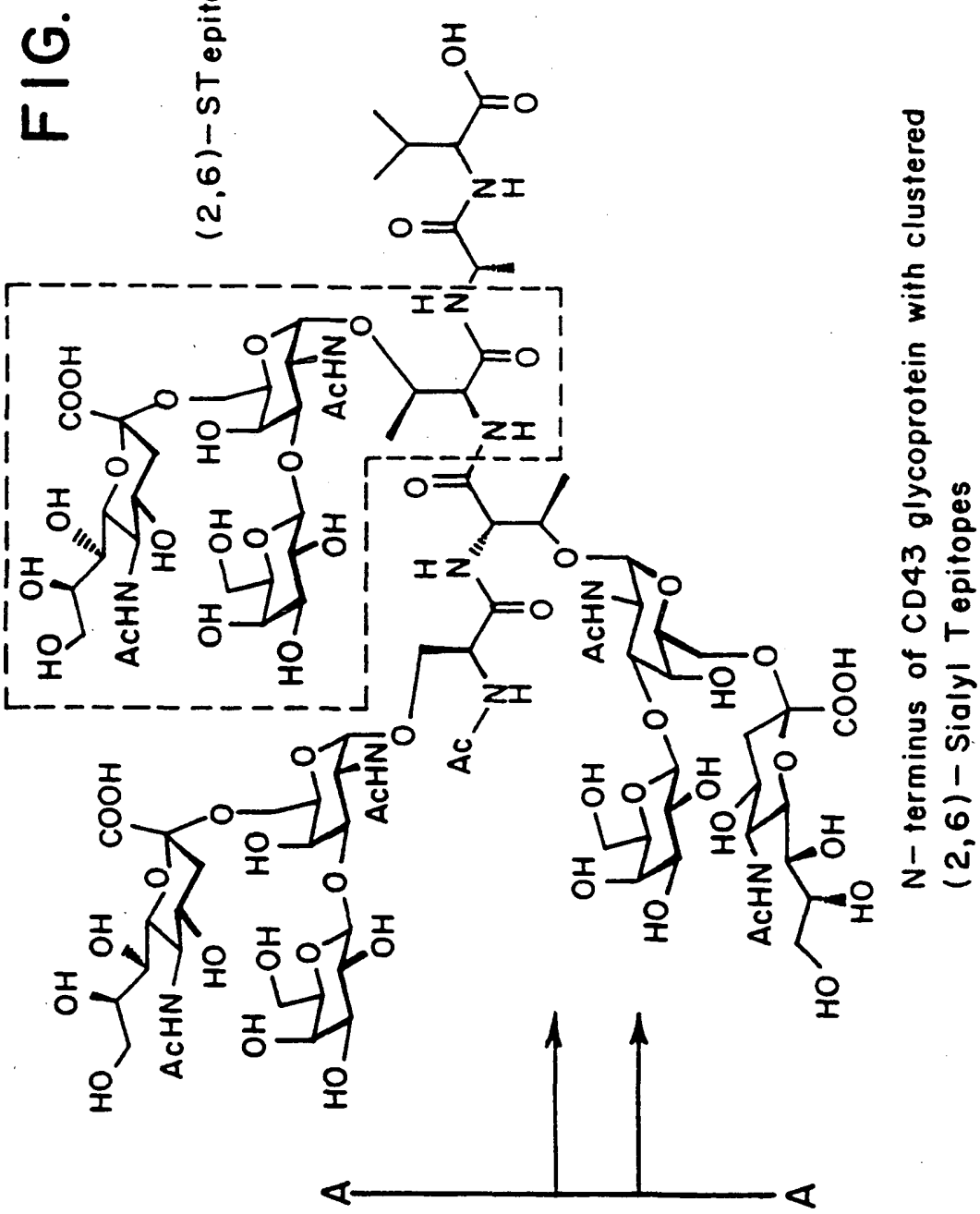

(2,6)-ST epitope
N-terminus of CD43 glycoprotein with clustered
(2,6)-Sialyl T epitopes

FIG. 3

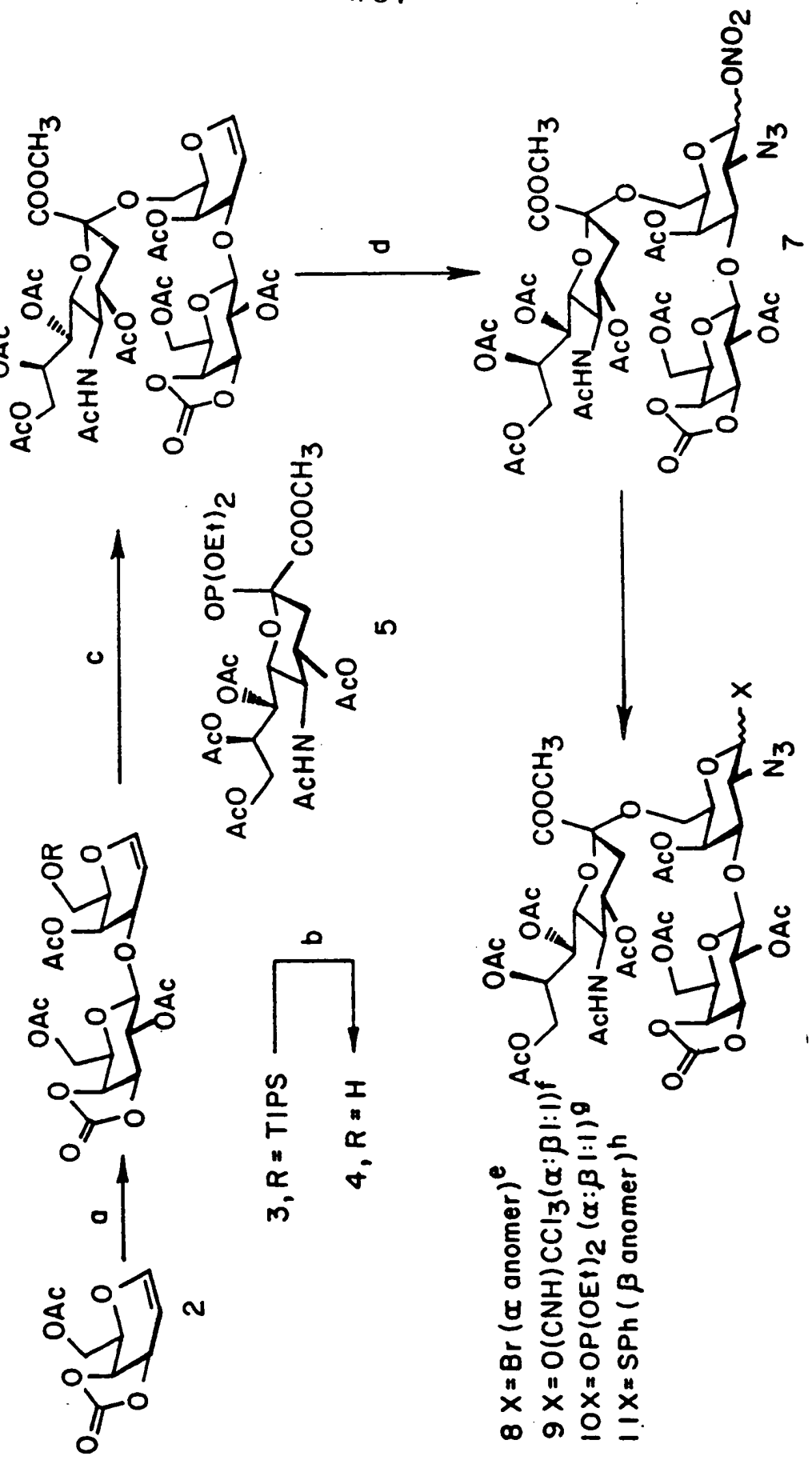

a
b
c
d
2
3, R = TIPS
4, R = H
5
7
8 X = Br (α anomer)e
9 X = O(CNH)CCl3(α:β 1:1)f
10 X = OP(OEt)2 (α:β 1:1)g
11 X = SPh (β anomer)h

FIG. 4A

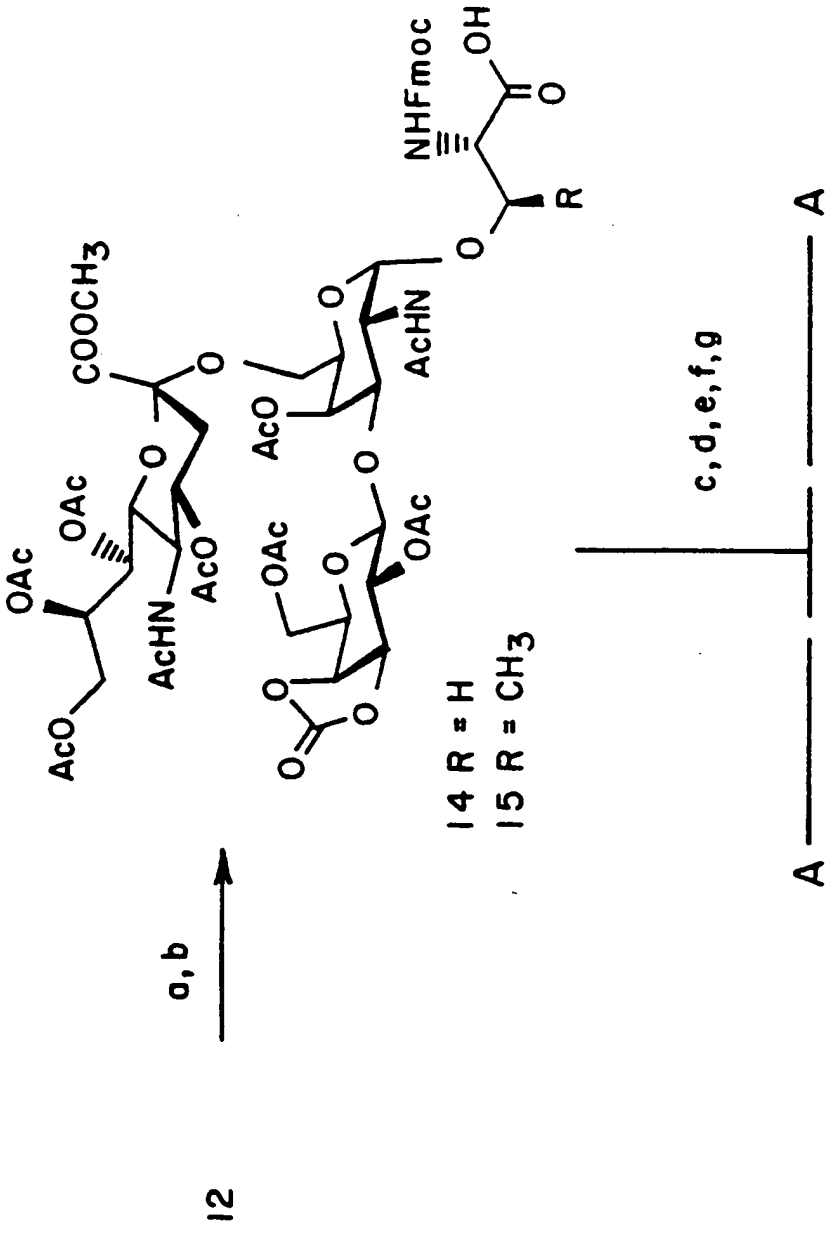


FIG. 4B

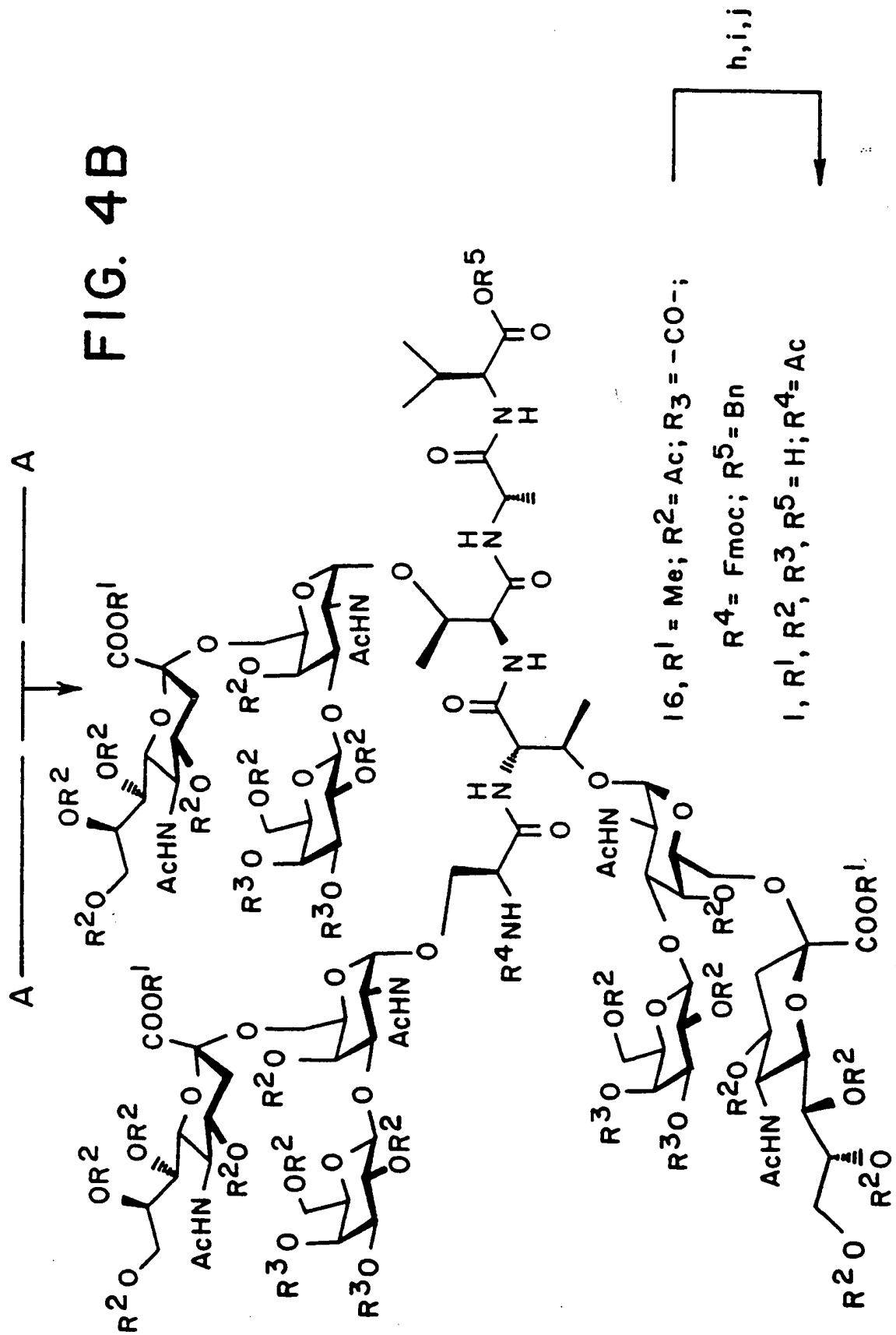


FIG. 5A

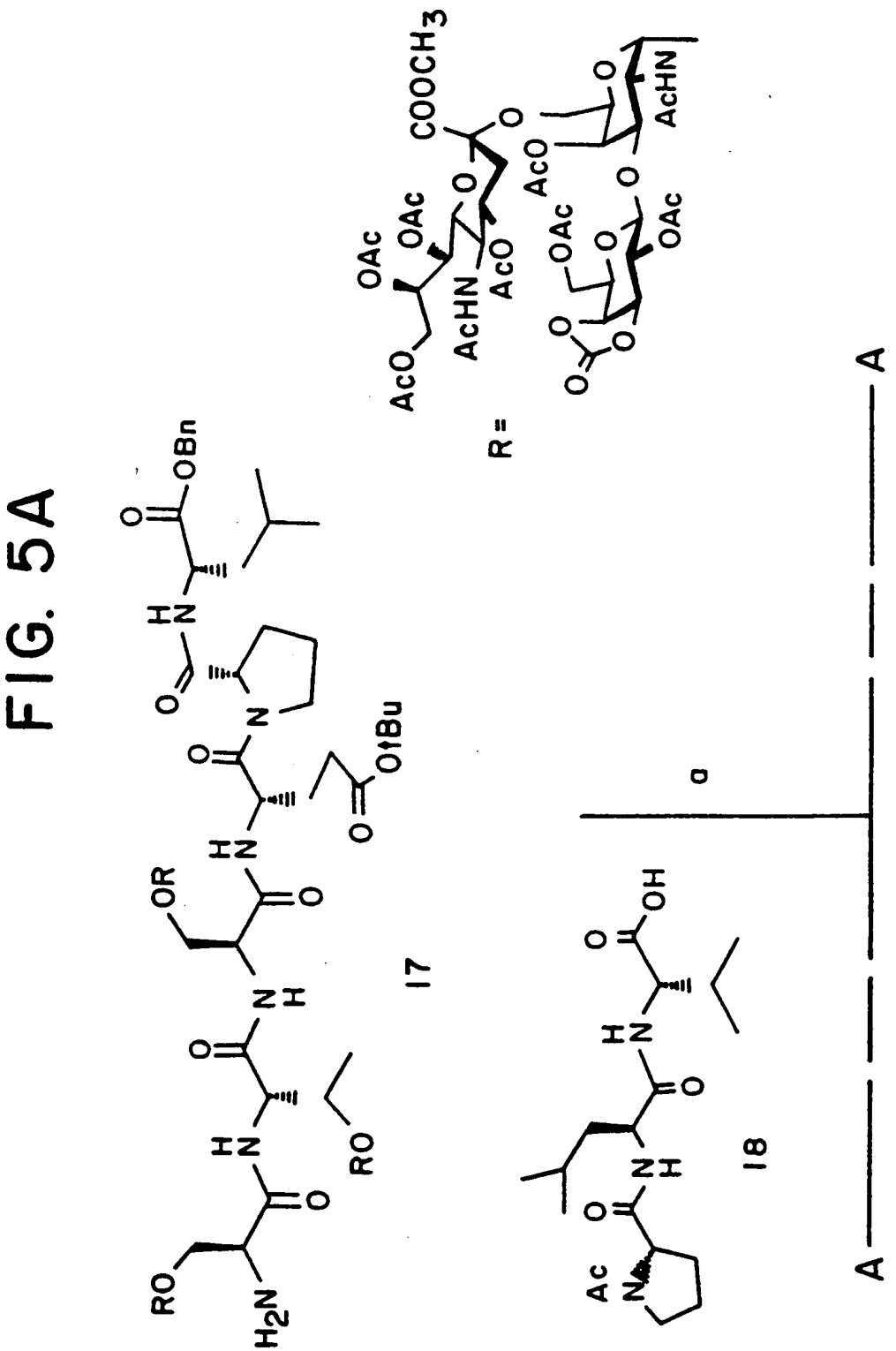


FIG. 5B

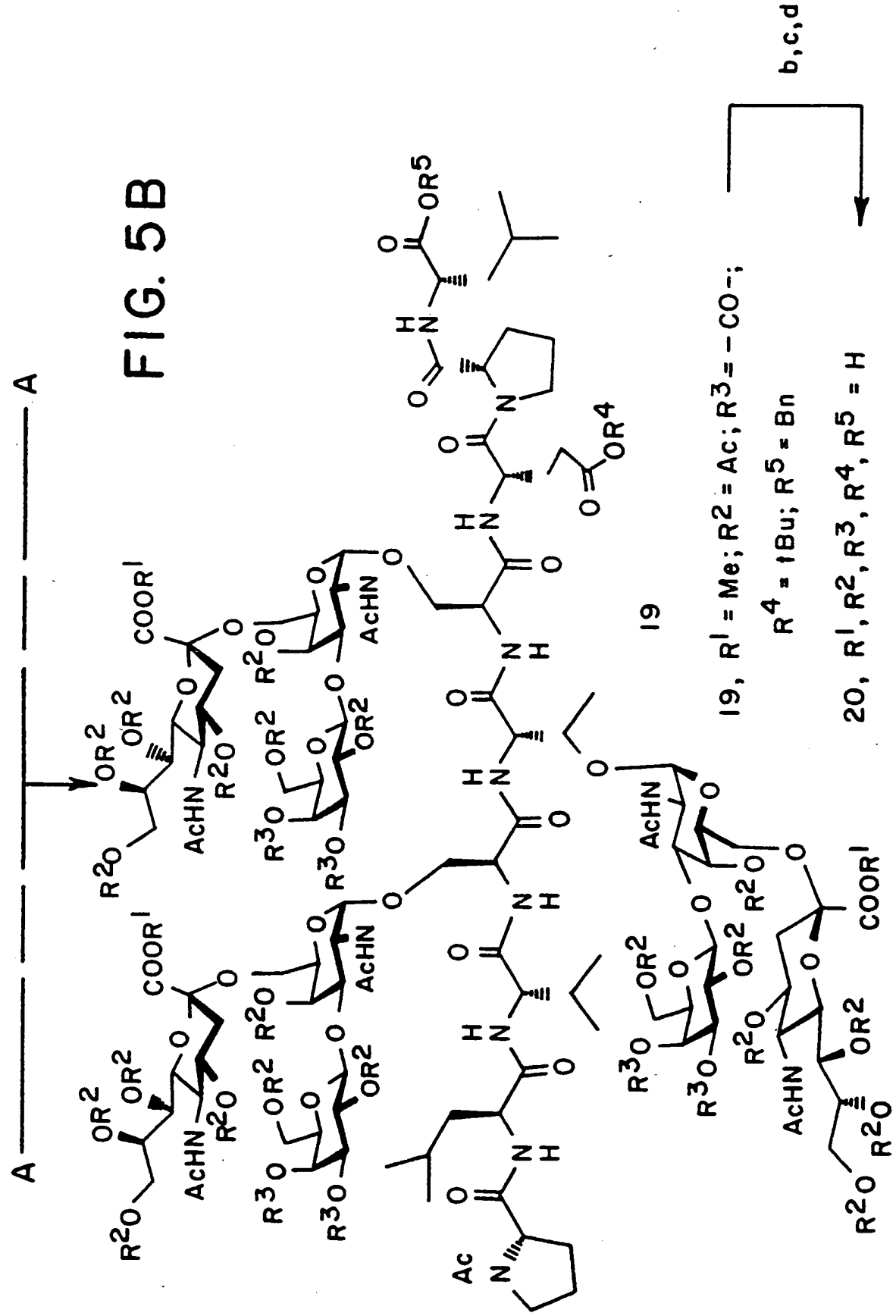


FIG. 6

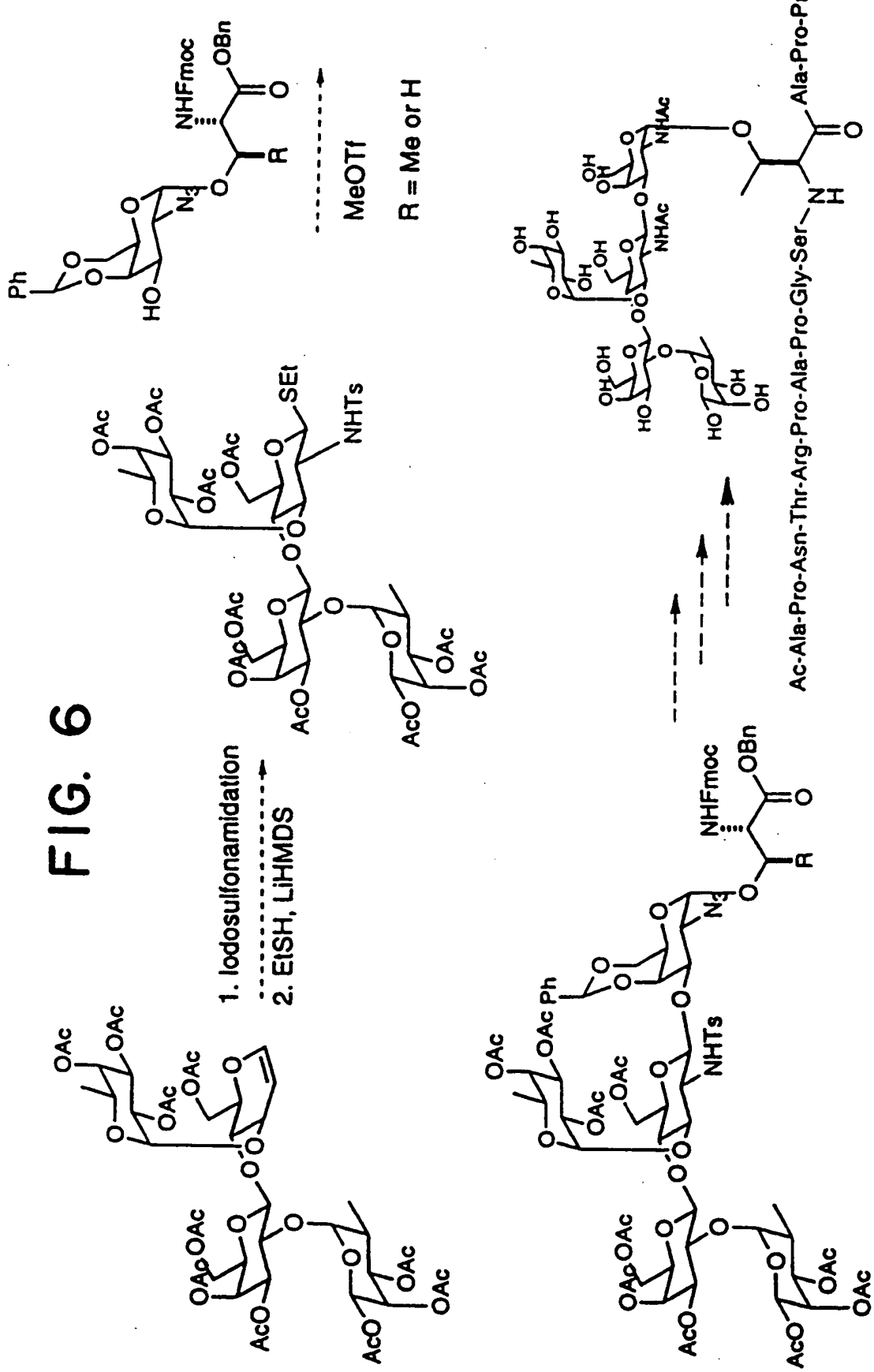

1. Iodosulfonamidation
2. EtSH, LiHMDS
MeOTf
R = Me or H
Ac-Ala-Pro-Asn-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Ala-Pro-Pro-Ala

FIG. 7A

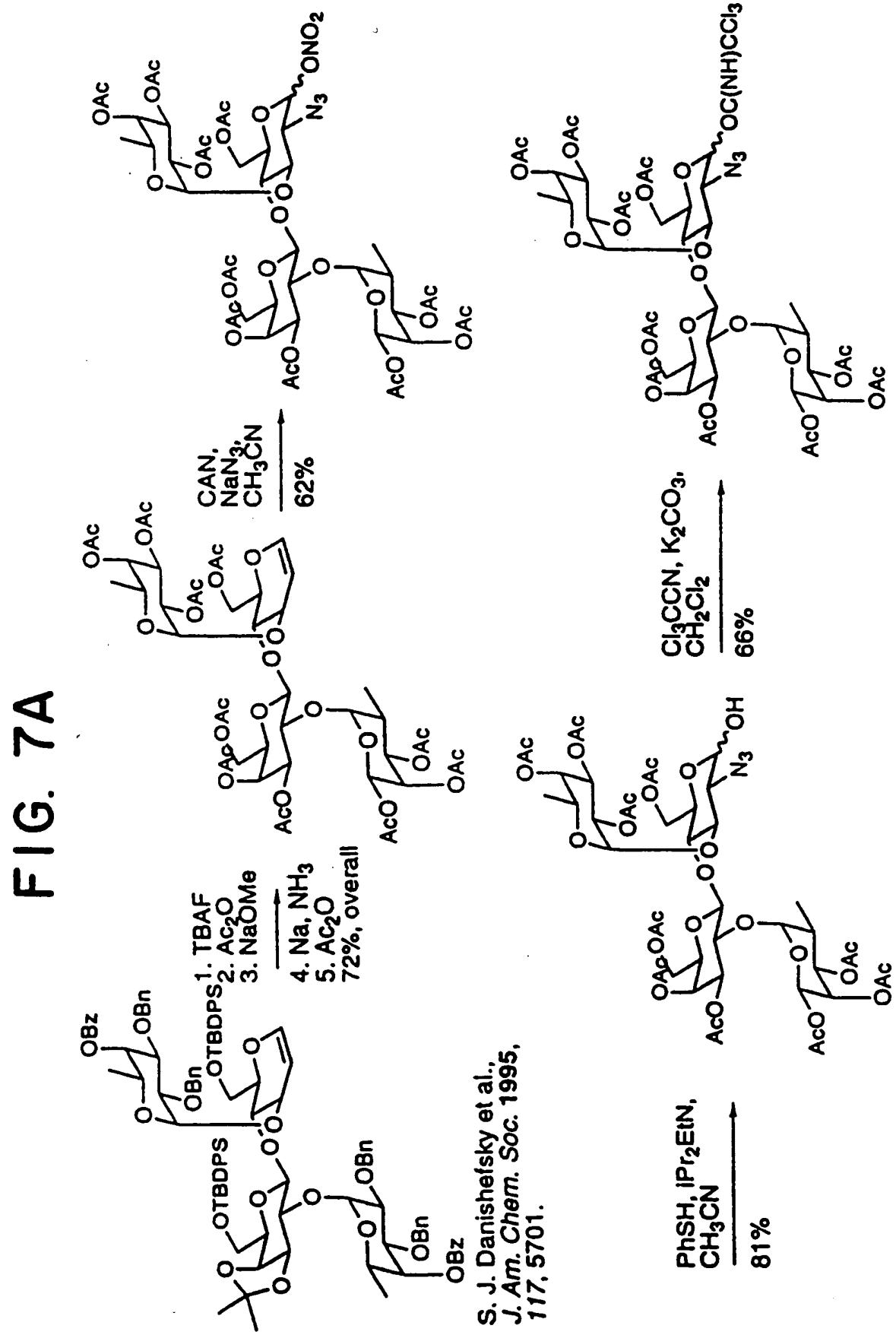

1. TBAF
2. Ac2O
3. NaOMe
4. Na, NH3
5. Ac2O
72%, overall
CAN, NaN3, CH3CN
62%
Cl3CCN, K2CO3, CH2Cl2
66%
PhSH, IPr2EtN, CH3CN
81%
S. J. Danishefsky et al., J. Am. Chem. Soc. 1995, 117, 5701.

FIG. 7B

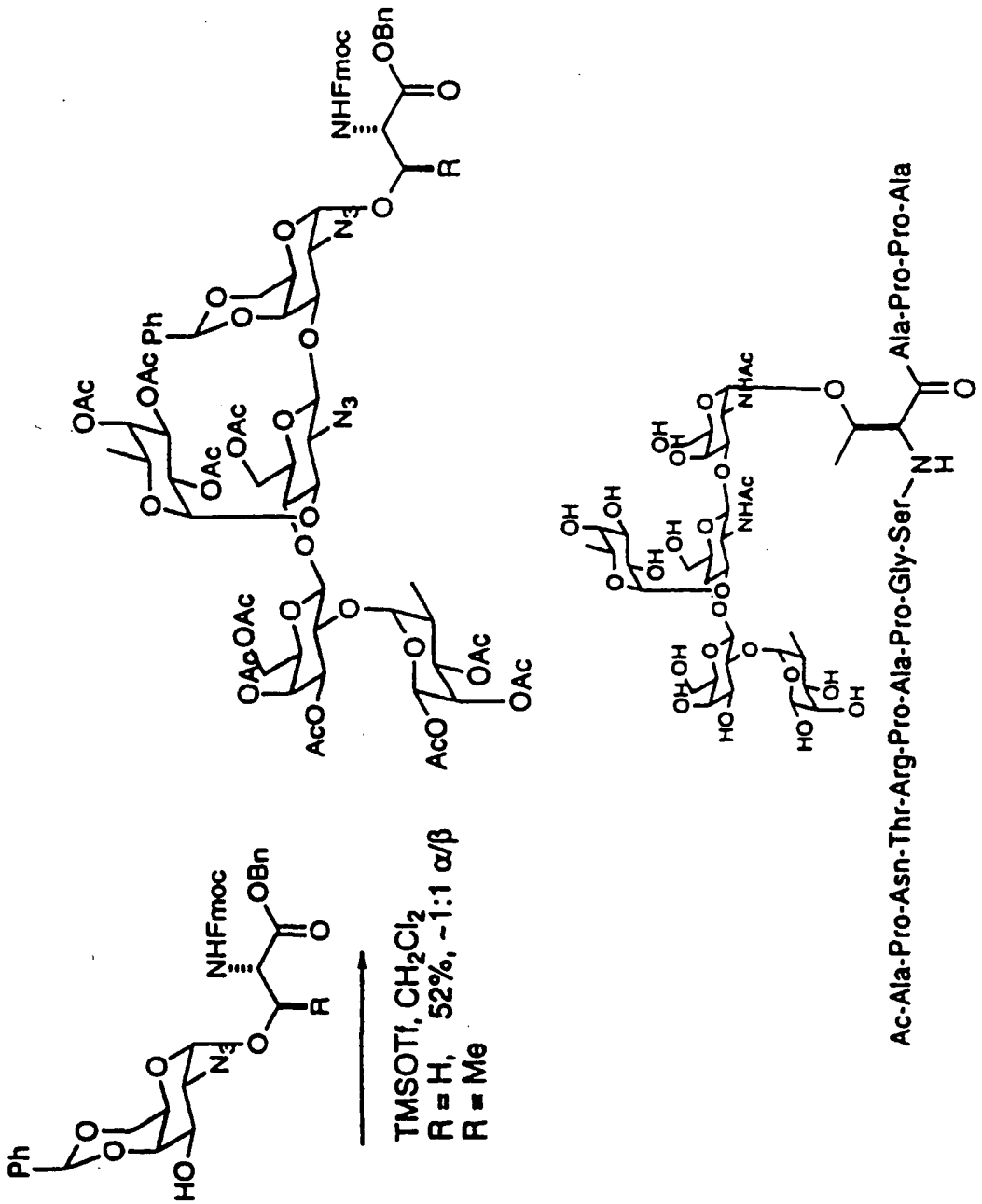
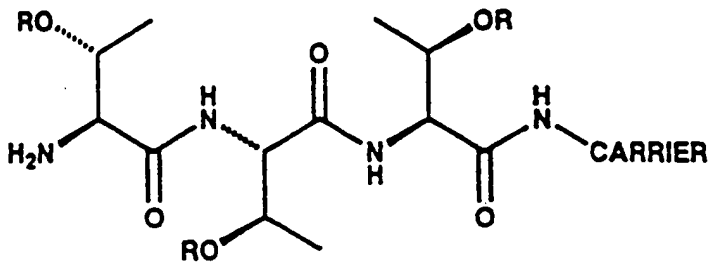

TMSOTf, CH_2Cl_2
R = H, 52%, ~1:1 α/β
R = Mθ
Ac-Ala-Pro-Asn-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Ala-Pro-Pro-Ala

FIG. 8A



1

FIG. 8B

ST_N 1a, R =

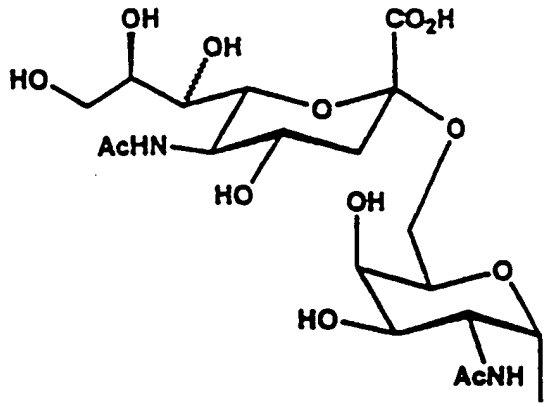


FIG. 8C

T(TF) 1b, R =

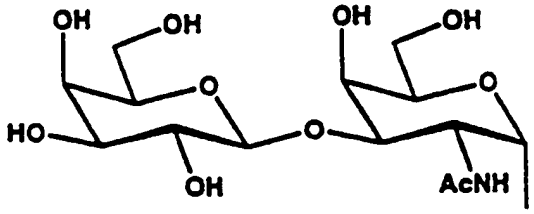


FIG. 8D

(2,3)ST 1c, R =

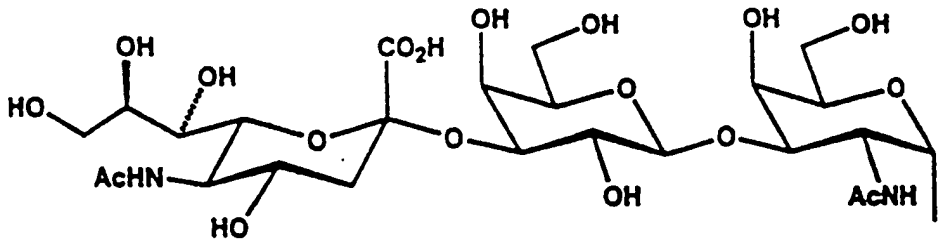


FIG. 8E

Glycophorine 1d, R =

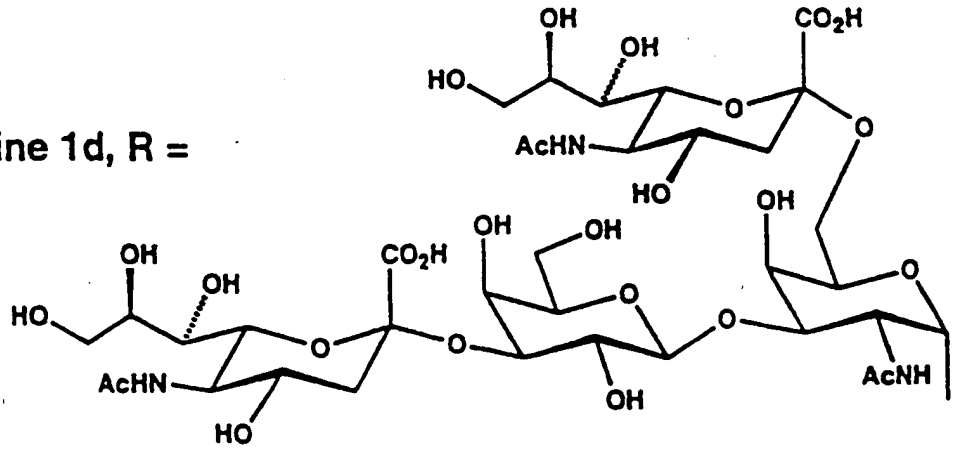
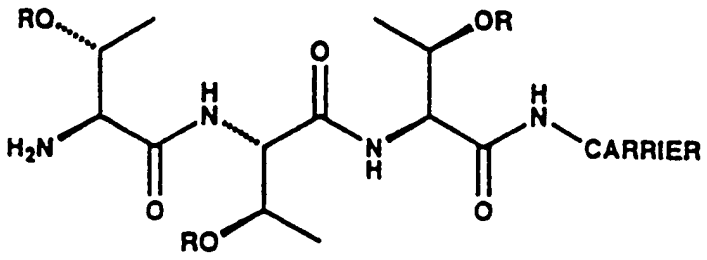


FIG. 9A



1

FIG. 9B

3-Le^y 1e, R =

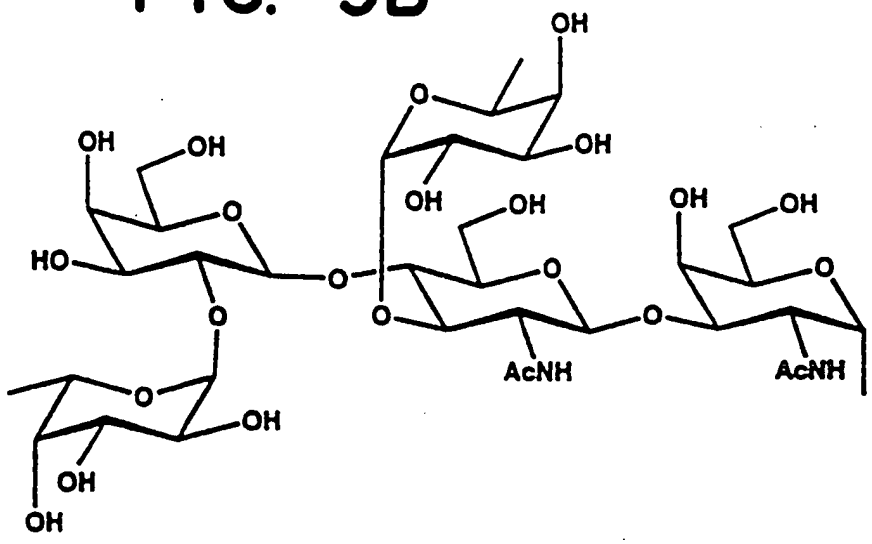


FIG. 9C

6-Le^y 1f, R =

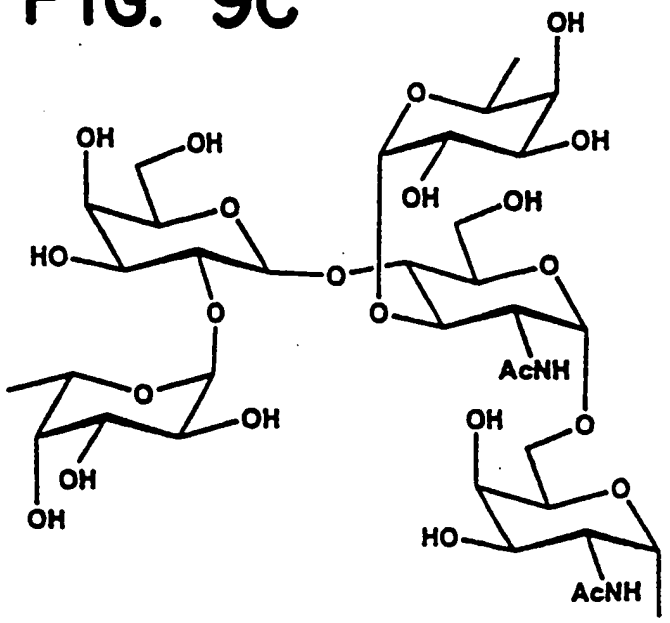


FIG. 10A

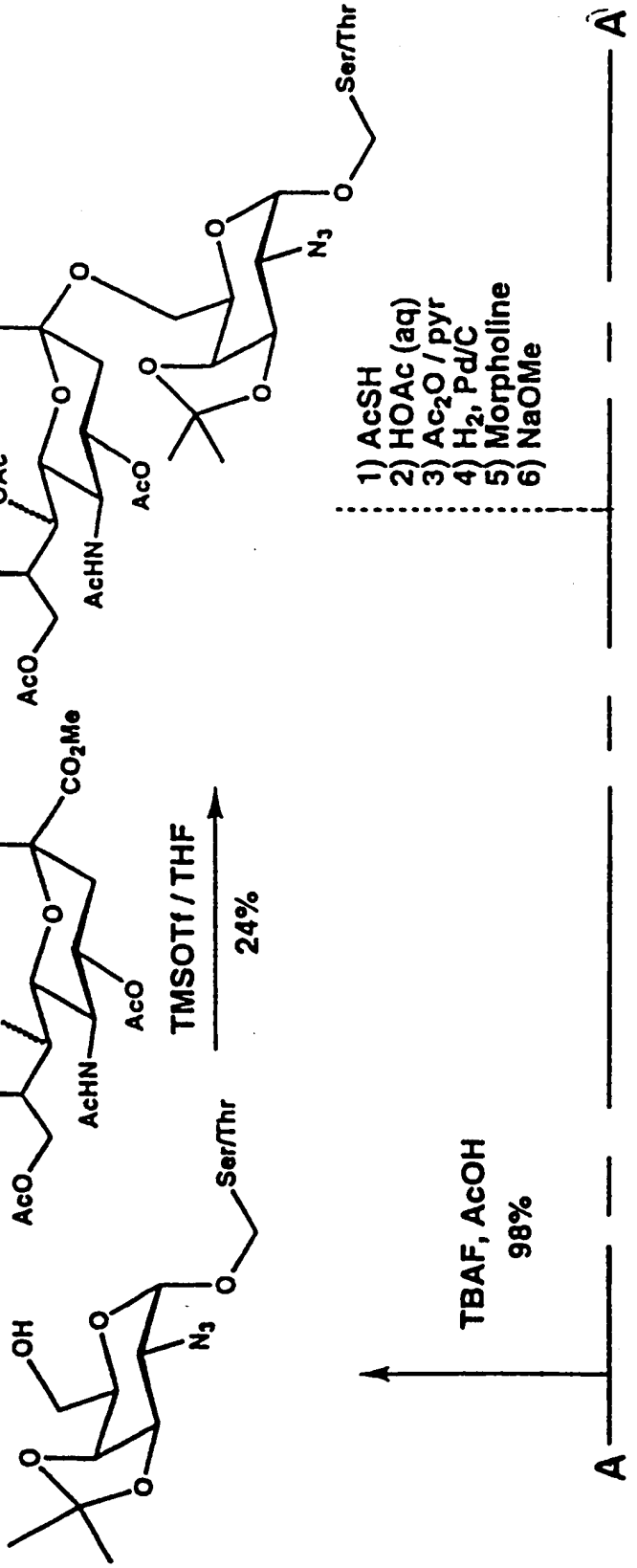

TBAF, AcOH
98%
TMSOTf / THF
24%
1) AcSH
2) HOAc (aq)
3) Ac_2O / pyr
4) H_2, Pd/C
5) Morpholine
6) NaOMe
A
A'
Ser/Thr
N_3
CO_2Me
OAc
AcO
AcHN
$OP(OBn)_2$
OH

FIG. 10B

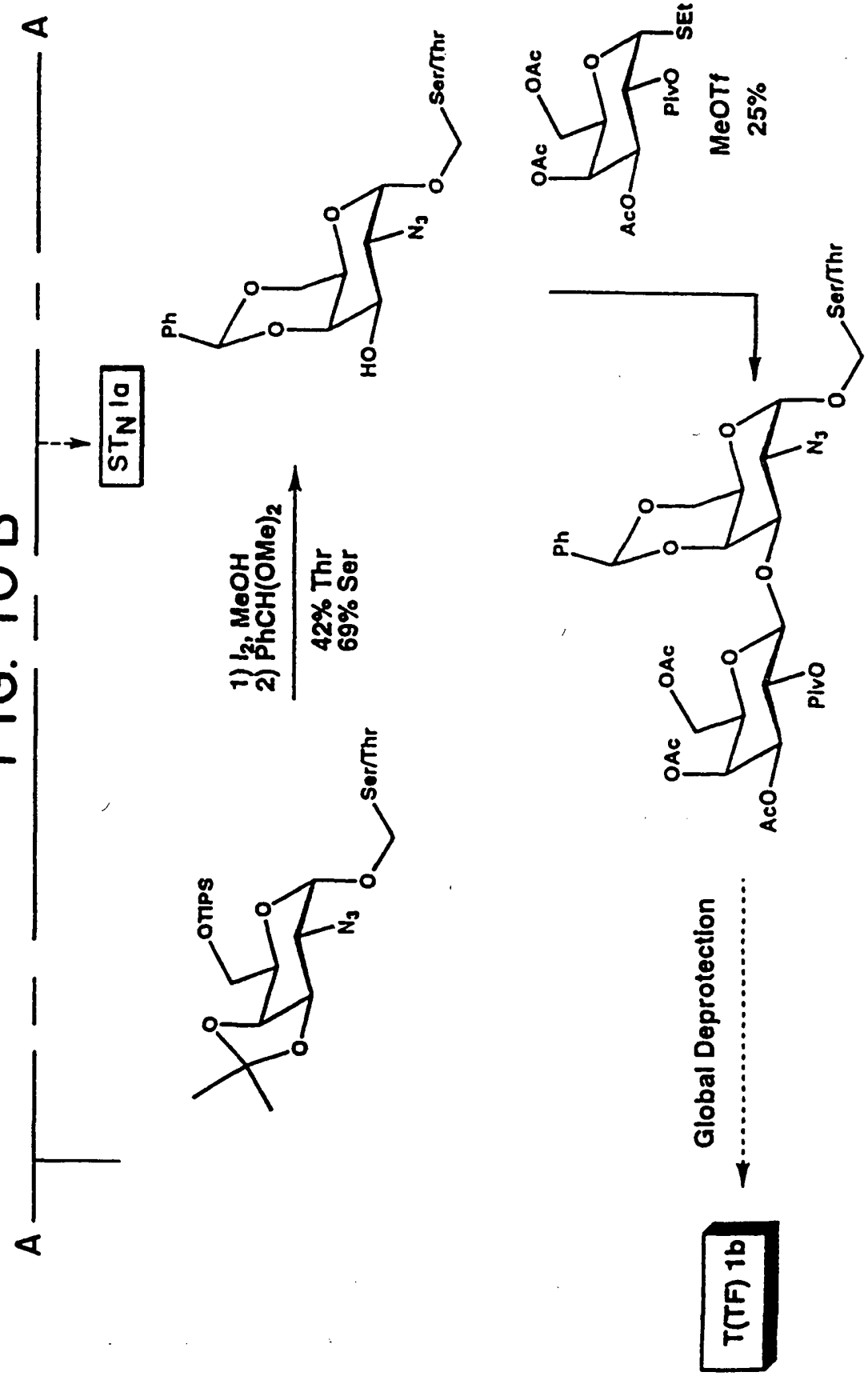

A
A
STN 1a
Ph
O
O
O
N3
HO
Ser/Thr
1) I2, MeOH
2) PhCH(OMe)2
42% Thr
69% Ser
OTIPS
O
O
O
N3
Ser/Thr
OAc
OAc
AcO
O
PivO
SEt
MeOTf
25%
Ph
O
O
O
N3
Ser/Thr
O
OAc
OAc
AcO
O
PivO
Global Deprotection
T(TF) 1b

FIG. 11A

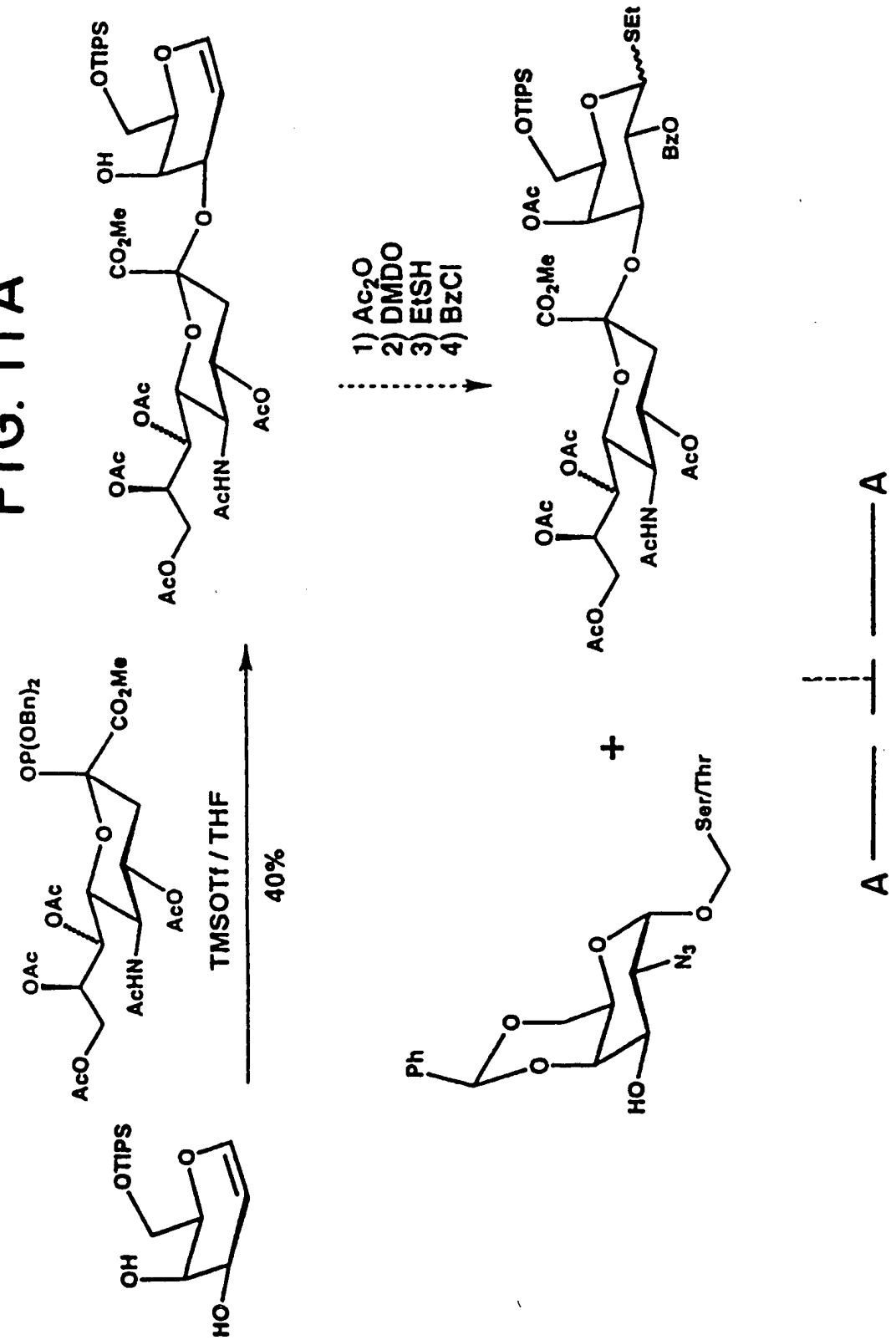

OTIPS
OH
HO
CO2Me
OP(OBn)2
OAc
AcHN
AcO
TMSOTf / THF
40%
1) Ac2O
2) DMDO
3) EtSH
4) BzCl
SEt
BzO
Ph
N3
Ser/Thr
A
A

FIG. 11B

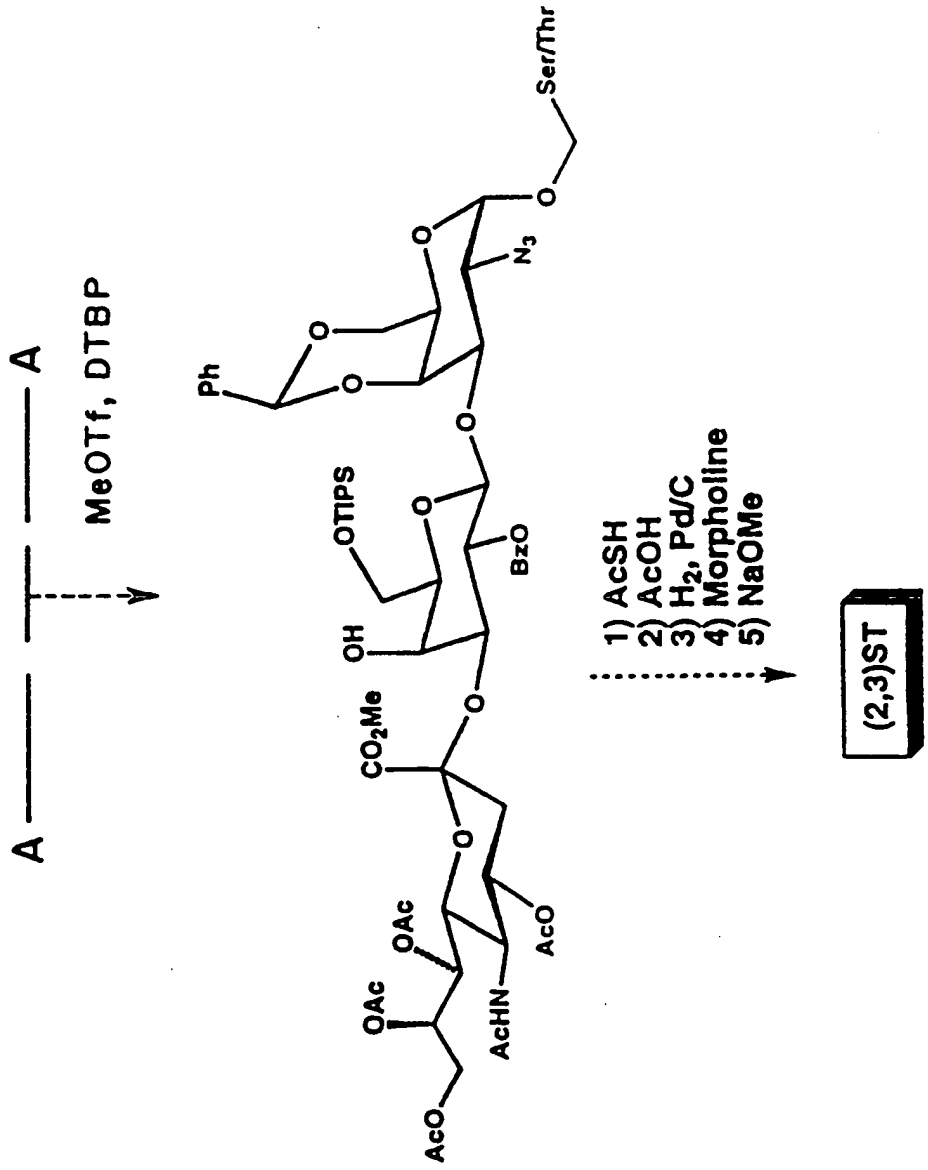

A
A
MeOTf, DTBP
Ph
Ser/Thr
N3
OTIPS
BzO
OH
CO2Me
OAc
OAc
AcHN
AcO
AcO
1) AcSH
2) AcOH
3) H2, Pd/C
4) Morpholine
5) NaOMe
(2,3)ST

FIG. 12A

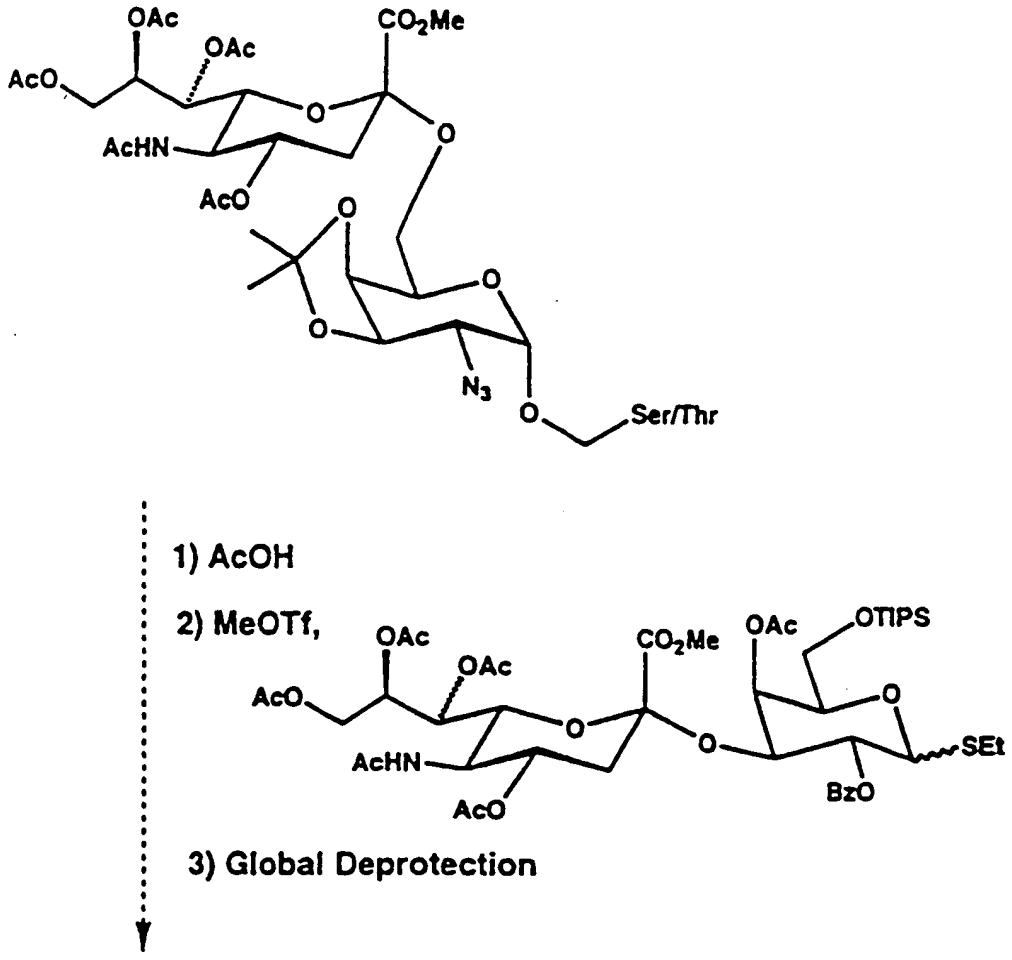

OAc
OAc
CO2Me
AcO
AcHN
AcO
O
N3
Ser/Thr
1) AcOH
2) MeOTf,
OTIPS
SEt
BzO
3) Global Deprotection
Glycophorine 1d
A
A

FIG. 12 B

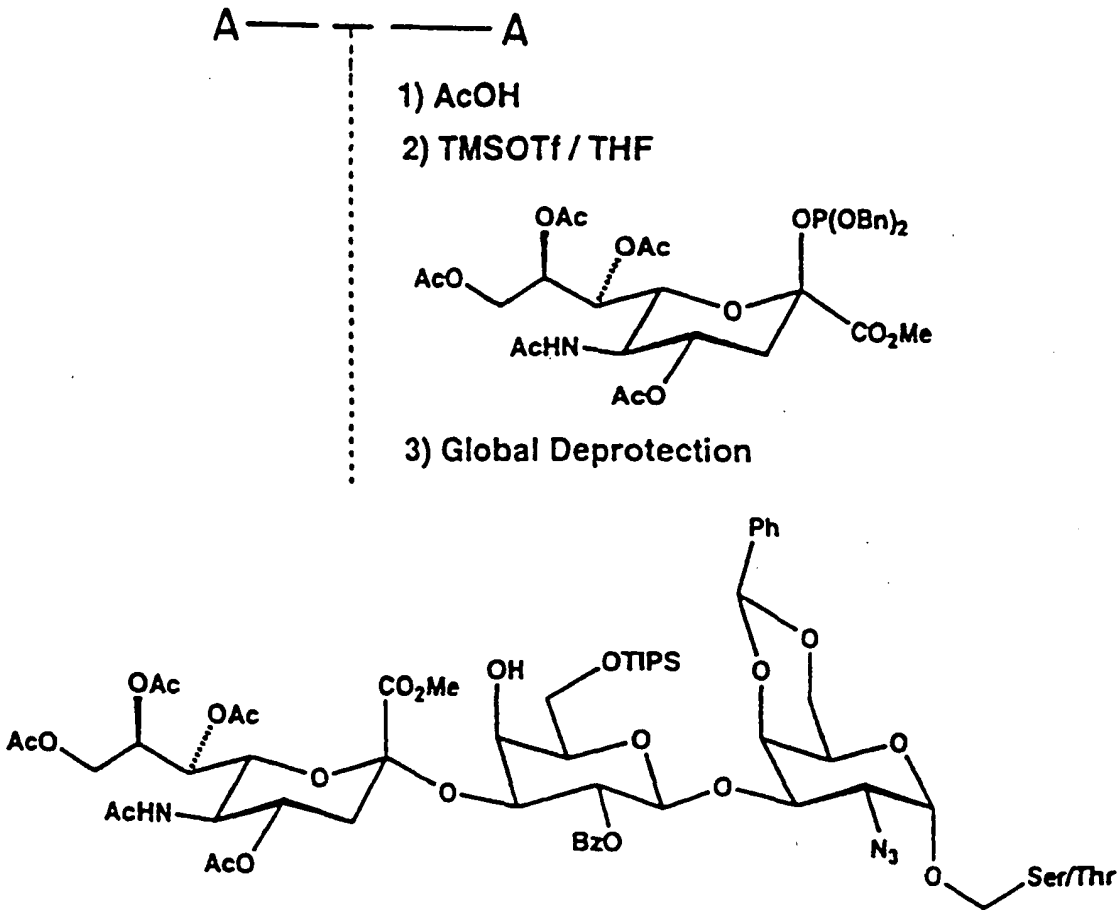

A
A
1) AcOH
2) TMSOTf / THF
OAc
OAc
OP(OBn)2
AcO
O
AcHN
CO2Me
AcO
3) Global Deprotection
Ph
O
OAc
OAc
CO2Me
OH
OTIPS
O
AcO
O
O
O
O
AcHN
O
O
AcO
BzO
N3
O
Ser/Thr

FIG. 13A

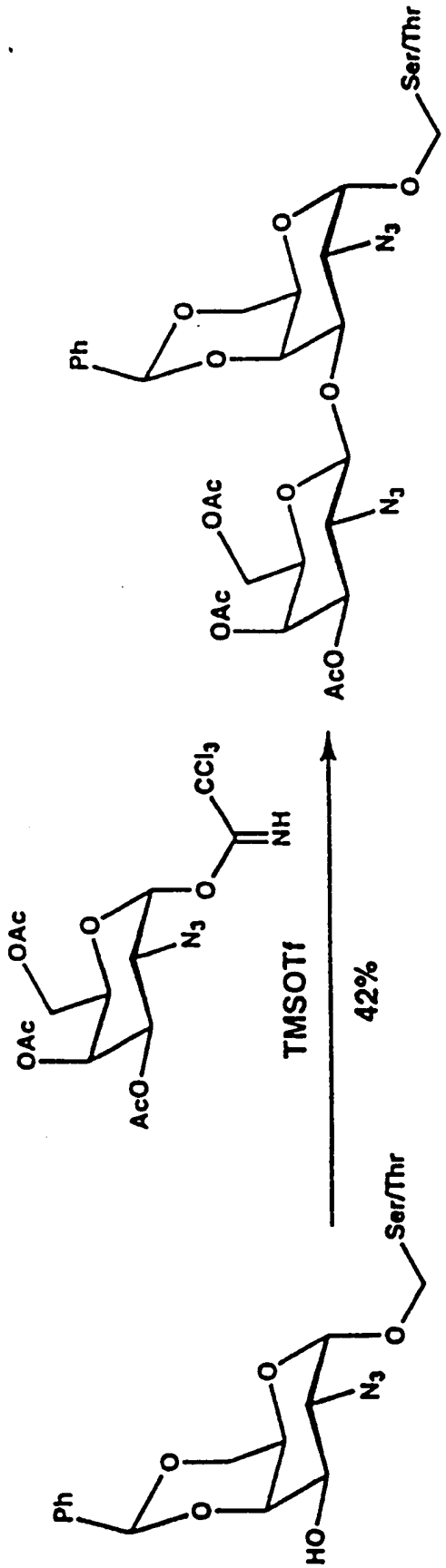

Ser/Thr
O
N3
O
Ph
O
O
OAc
O
N3
OAc
AcO
CCl3
NH
O
OAc
O
N3
OAc
AcO
TMSOTf
42%
Ser/Thr
O
N3
O
Ph
O
HO

FIG. 13B

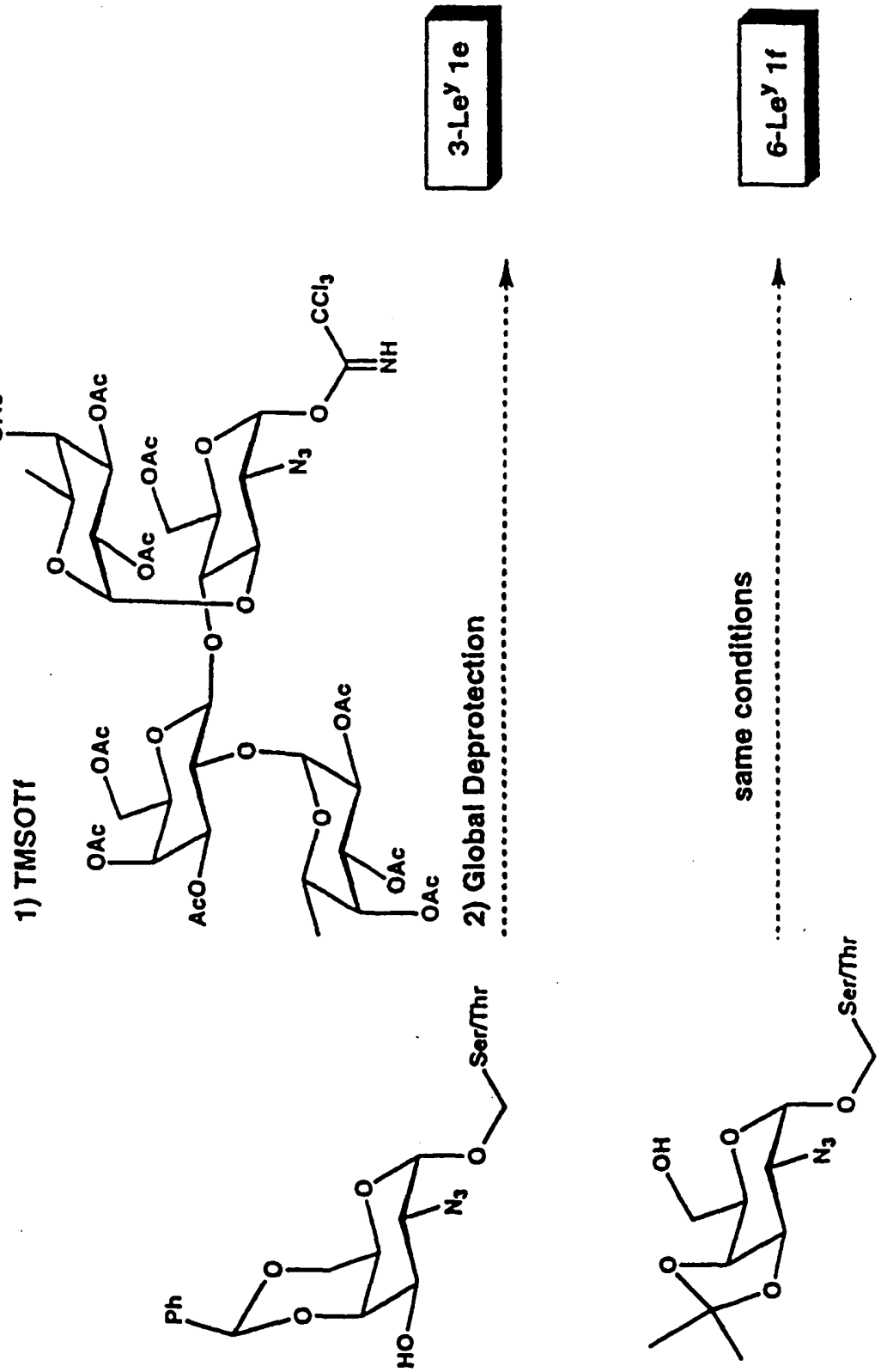

1) TMSOTf
2) Global Deprotection
3-Le^y 1e
same conditions
6-Le^y 1f
Ser/Thr
Ser/Thr

FIG. 14A

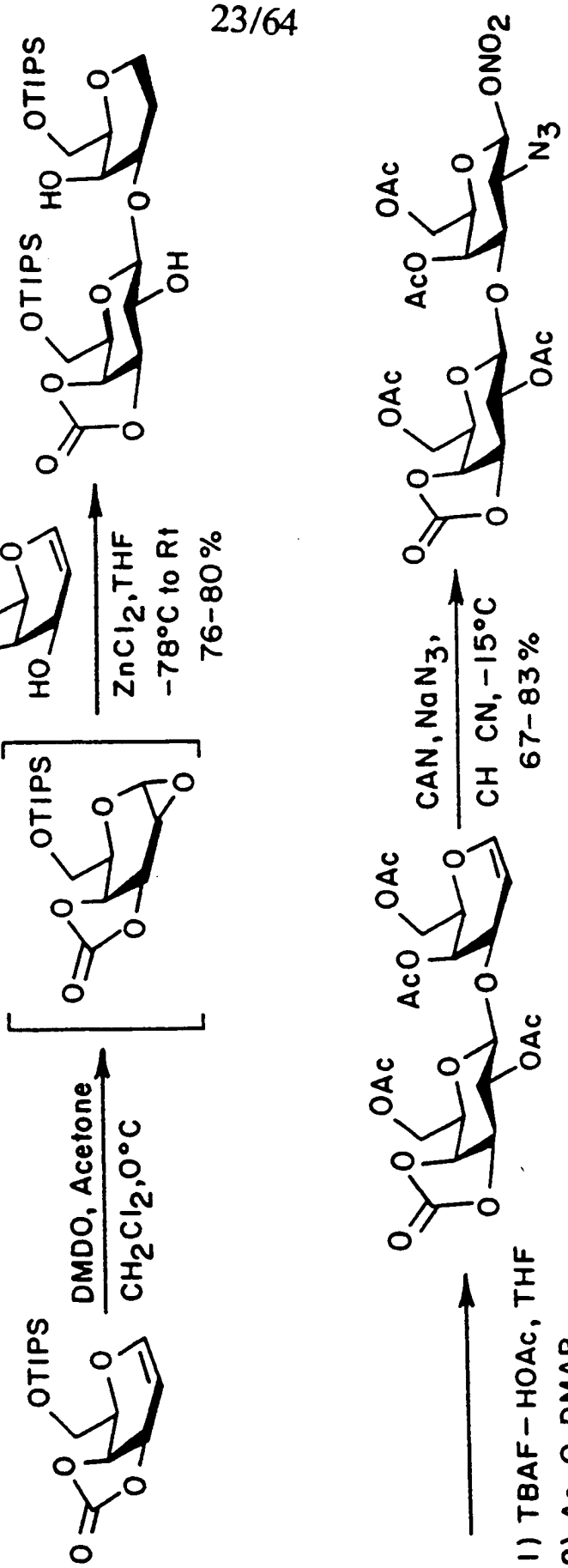

OTIPS
DMDO, Acetone
CH_2Cl_2, 0°C
OTIPS
HO
HO
$ZnCl_2$, THF
-78°C to RT
76-80%
OTIPS
HO
OTIPS
OH
1) TBAF-HOAc, THF
2) Ac_2O, DMAP
TEA, CH_2Cl_2
87%
OAc
AcO
OAc
OAc
CAN, NaN_3,
CH CN, -15°C
67-83%
OAc
AcO
N_3
ONO_2
OAc

FIG. 14B

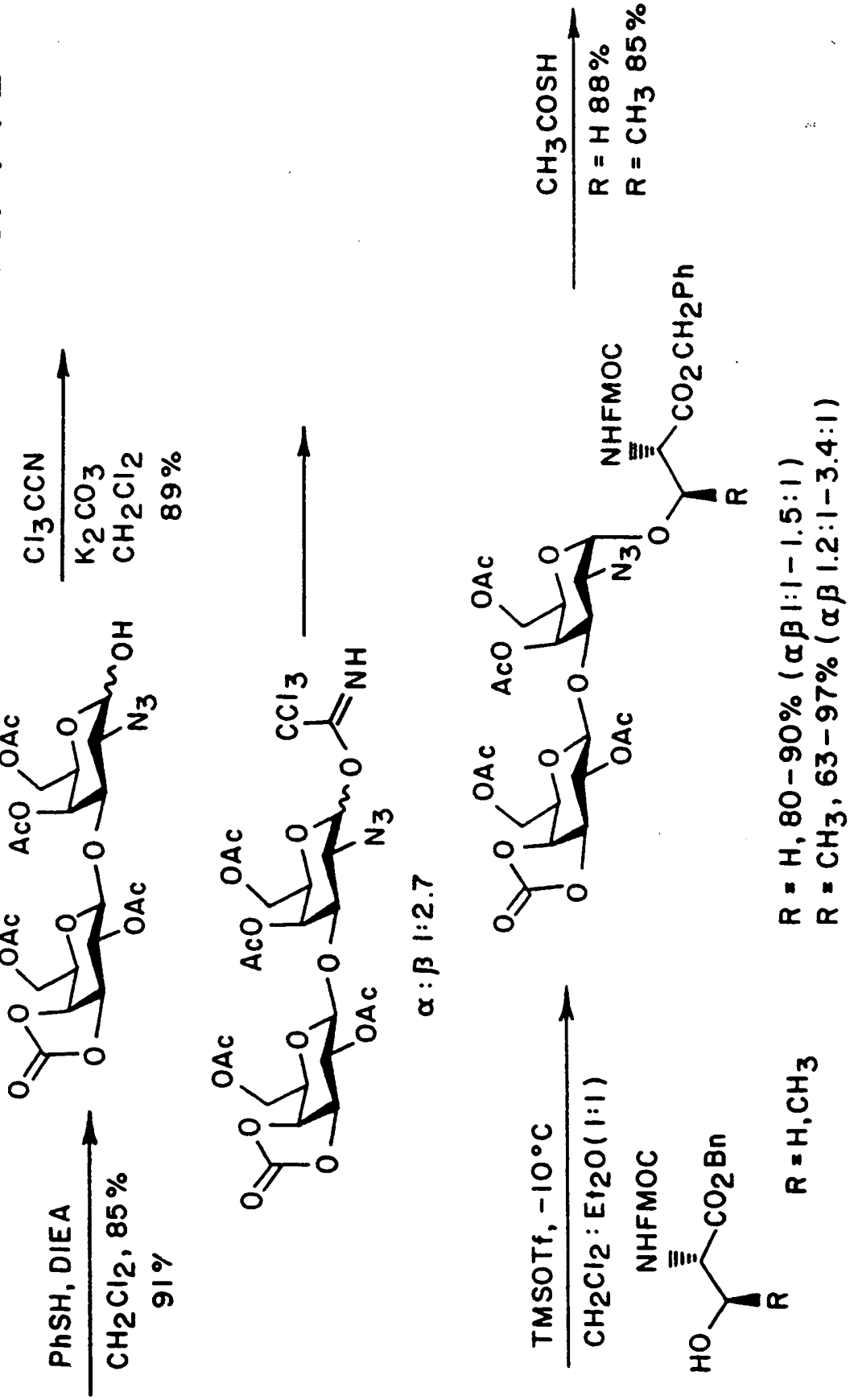

PhSH, DIEA
CH2Cl2, 85%
91%
Cl3CCN
K2CO3
CH2Cl2
89%
α:β 1:2.7
TMSOTf, -10°C
CH2Cl2 : Et2O (1:1)
NHFMOC
CO2Bn
R
HO
R = H, CH3
CH3COSH
R = H 88%
R = CH3 85%
NHFMOC
CO2CH2Ph
R = H, 80-90% (αβ 1:1-1.5:1)
R = CH3, 63-97% (αβ 1.2:1-3.4:1)

FIG. 14C

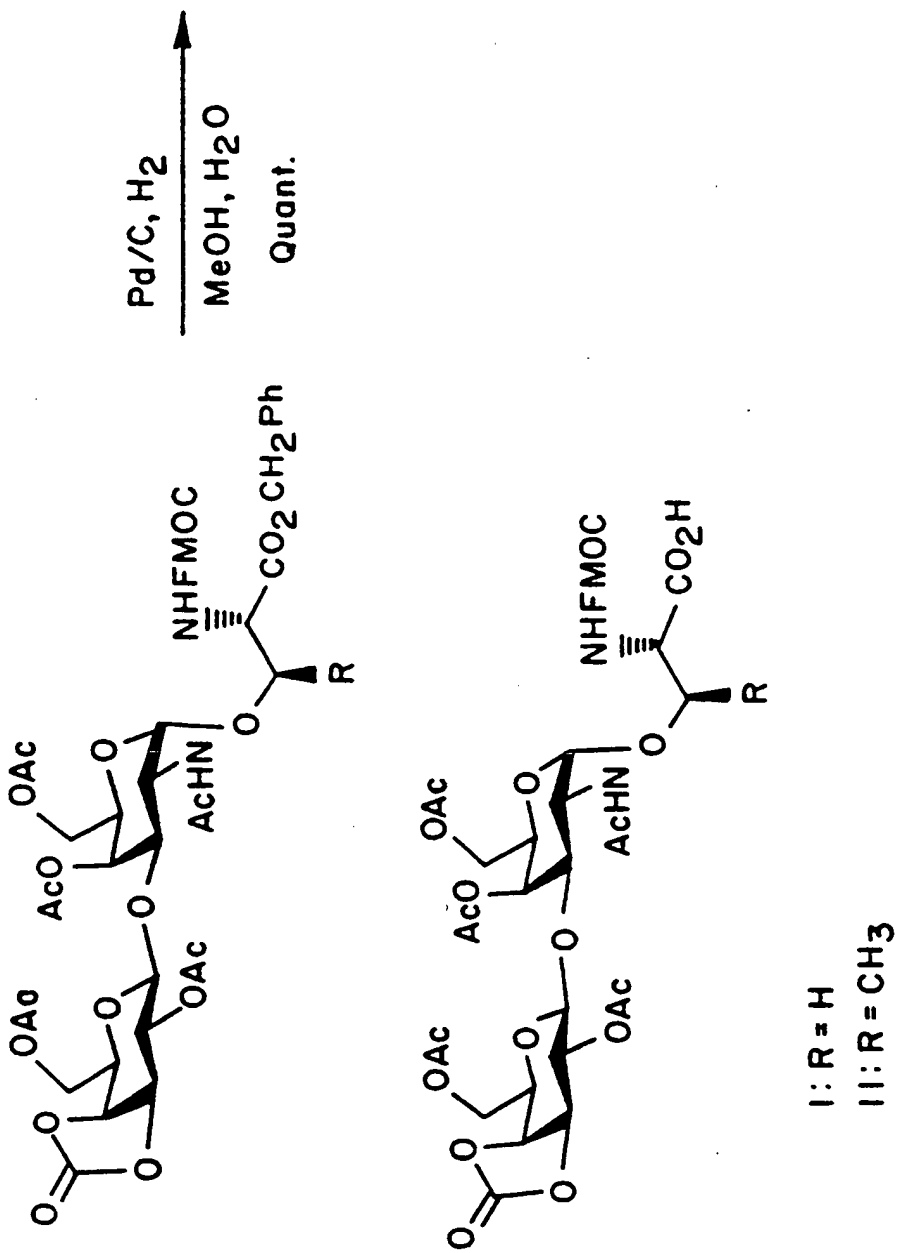

Pd/C, H2
MeOH, H2O
Quant.
NHFMOC
CO2CH2Ph
R
AcHN
AcO
OAc
O
NHFMOC
CO2H
R
AcHN
AcO
OAc
O
I: R = H
II: R = CH3

FIG. 15A

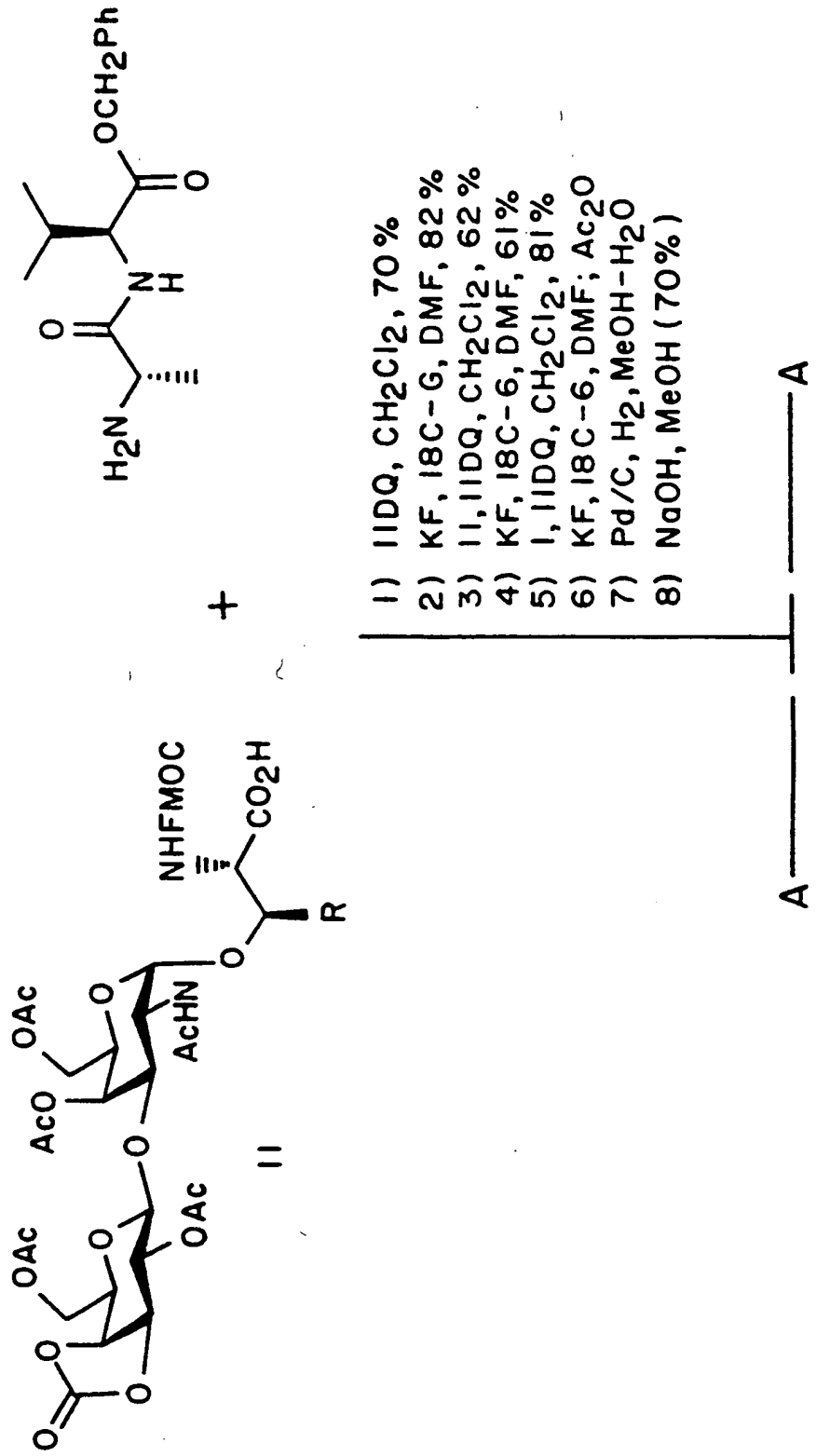

OCH2Ph
O
NH
O
H2N
+
NHFMOC
CO2H
R
O
AcHN
OAc
AcO
O
OAc
OAc
O
O
O
O
II
1) IIDQ, CH2Cl2, 70%
2) KF, 18C-6, DMF, 82%
3) II, IIDQ, CH2Cl2, 62%
4) KF, 18C-6, DMF, 61%
5) I, IIDQ, CH2Cl2, 81%
6) KF, 18C-6, DMF; Ac2O
7) Pd/C, H2, MeOH-H2O
8) NaOH, MeOH (70%)
A
A

FIG. 15B

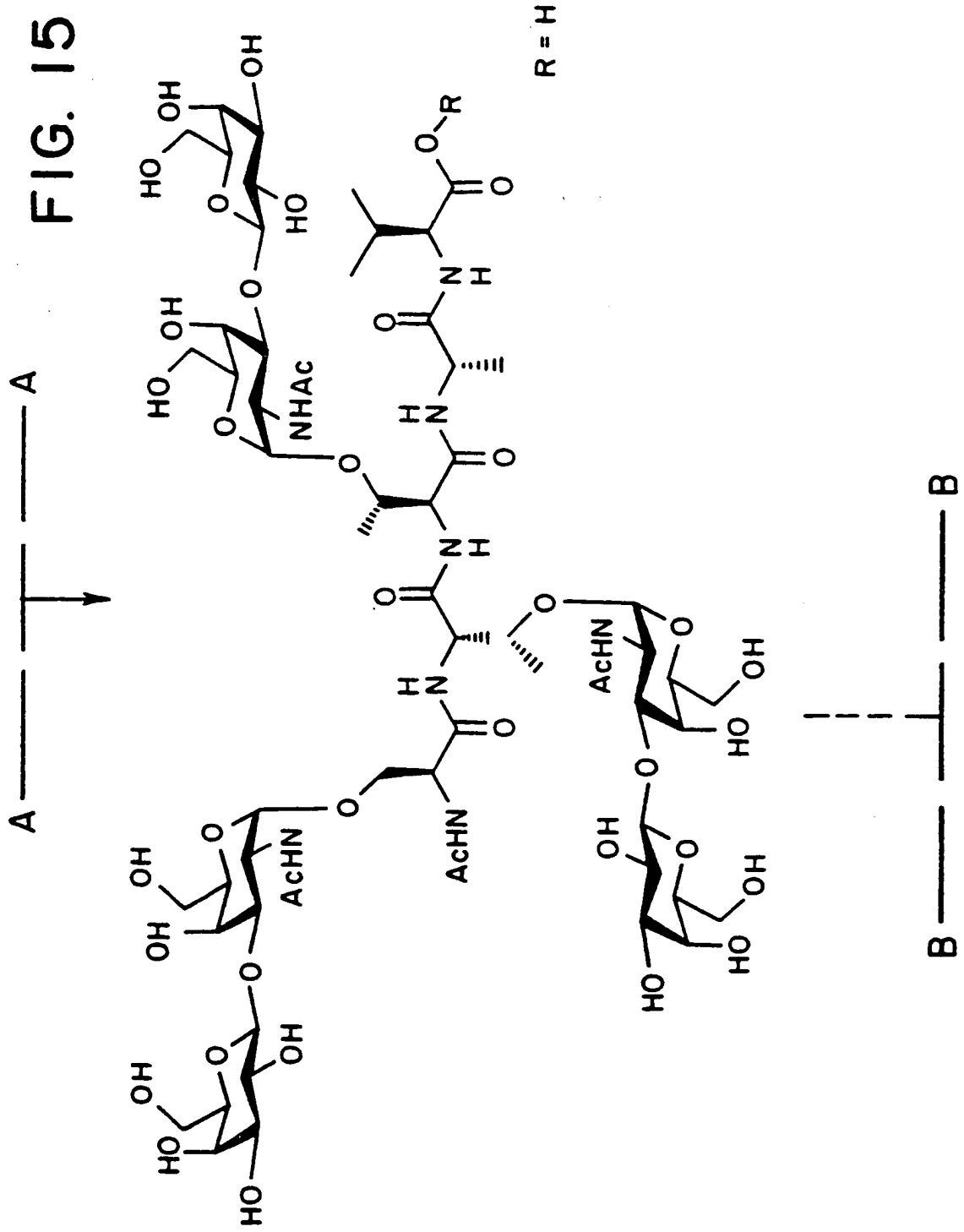
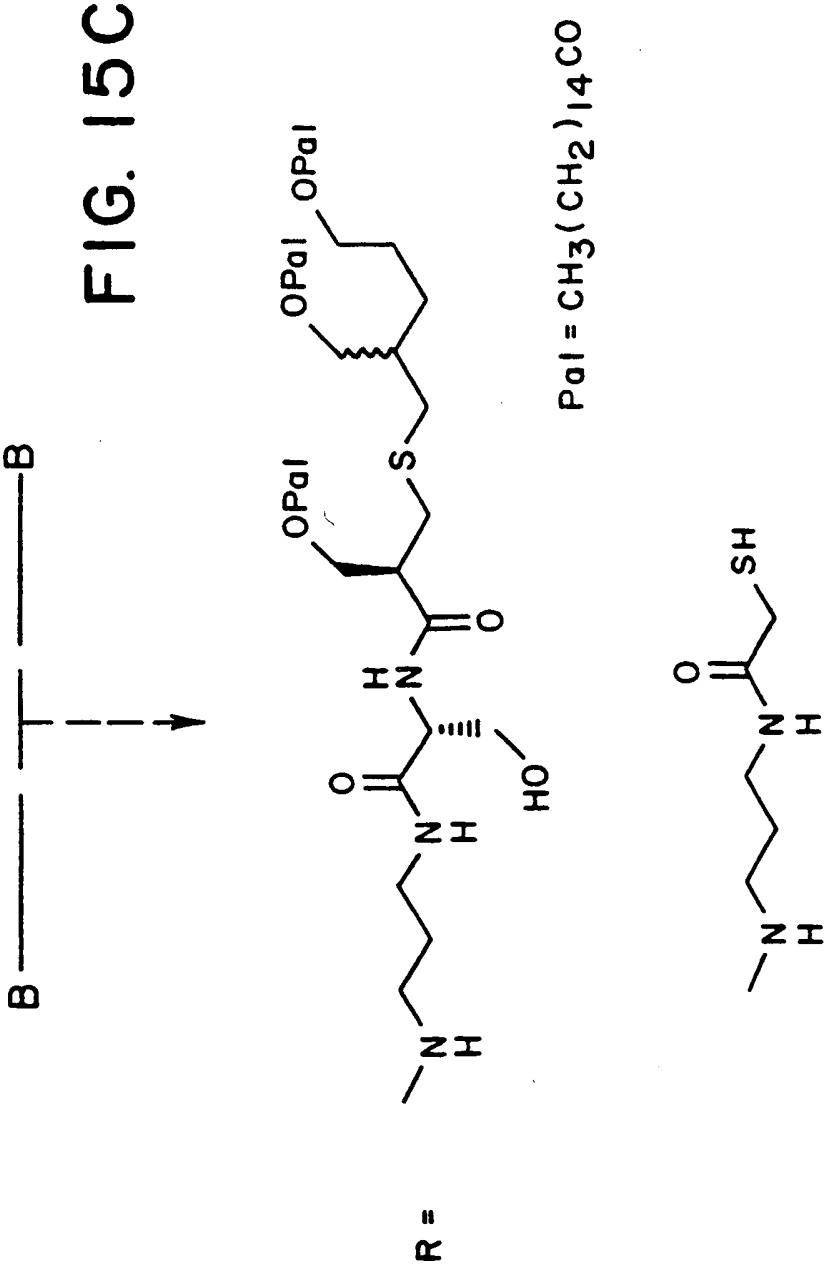


FIG. 15C



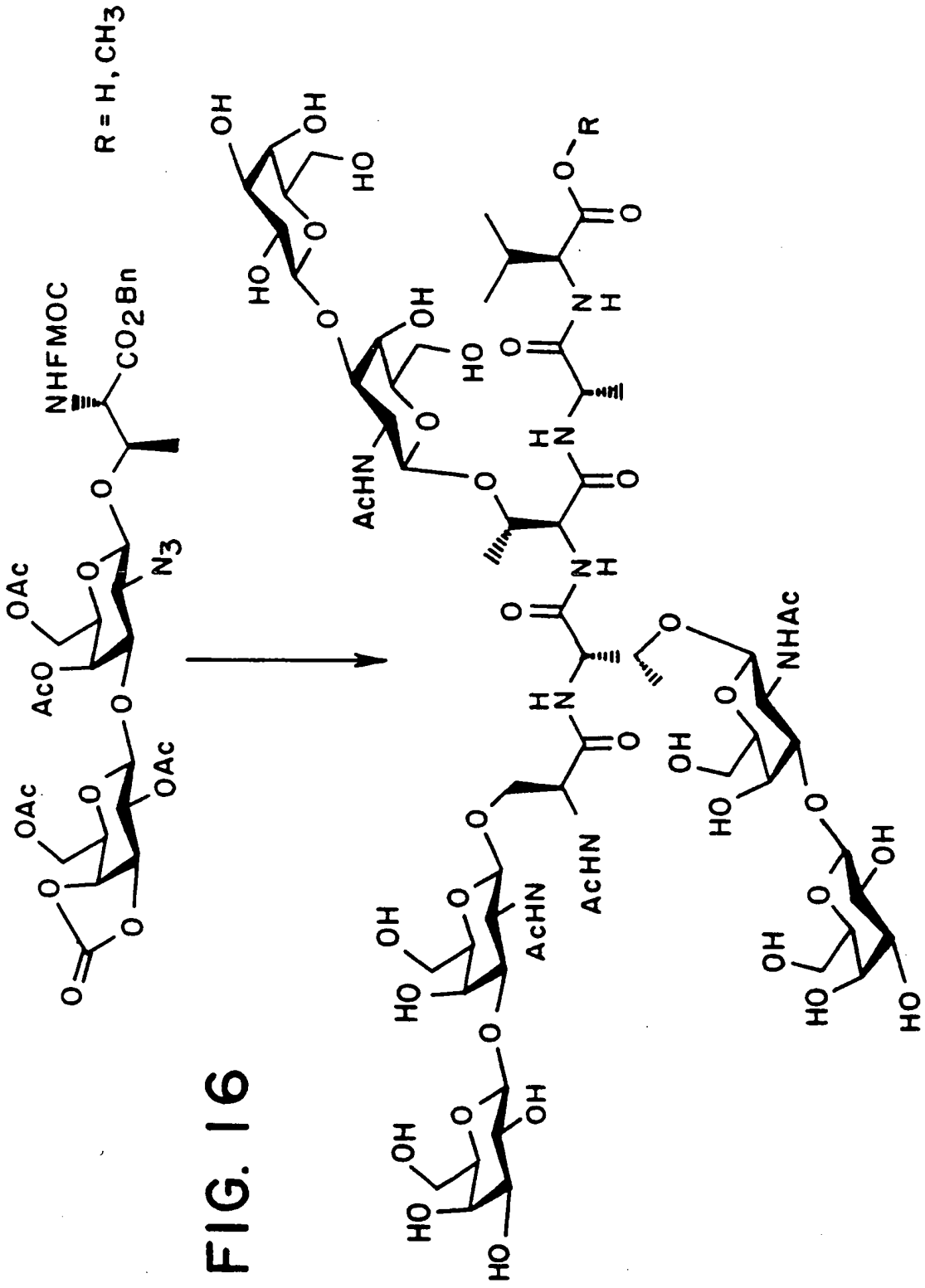


FIG. 16

FIG. 17A

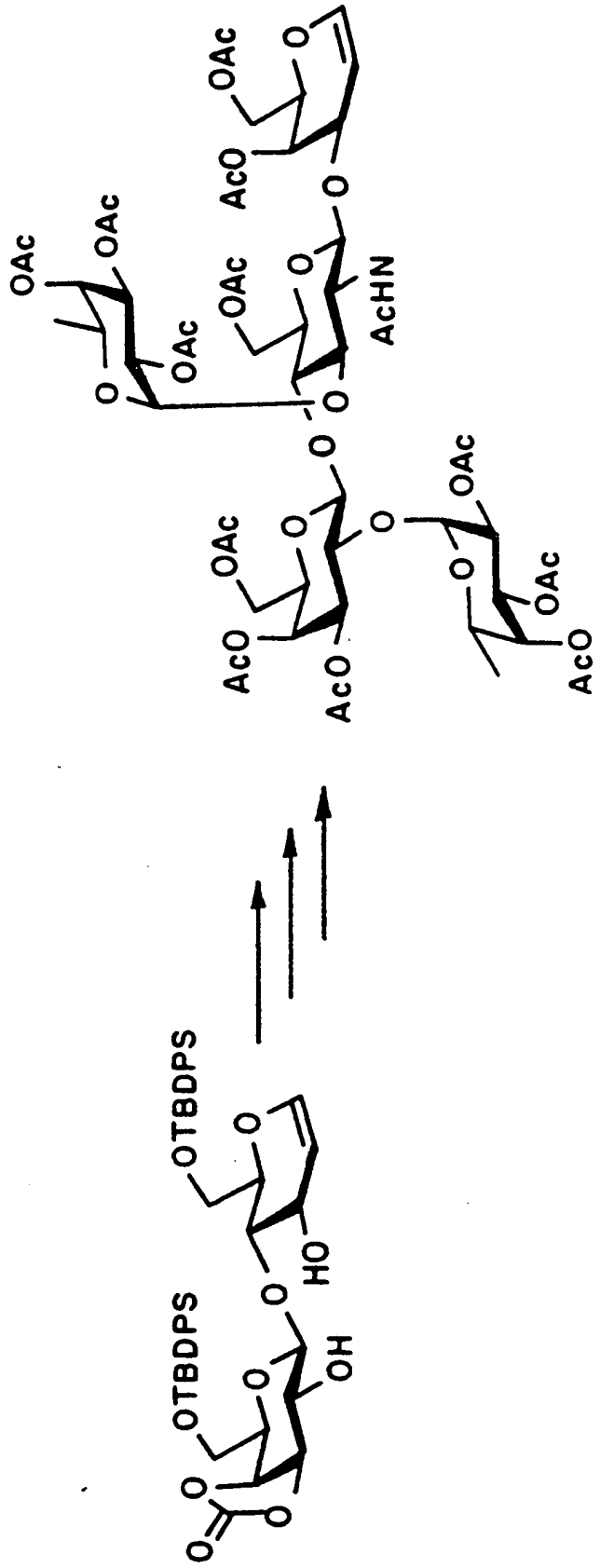

OAc
OAc
OAc
O
OAc
AcO
O
AcHN
O
O
OAc
AcO
O
O
OAc
AcO
O
OAc
AcO
OTBDPS
HO
O
OTBDPS
O
OH
O
O
O

FIG. 17B

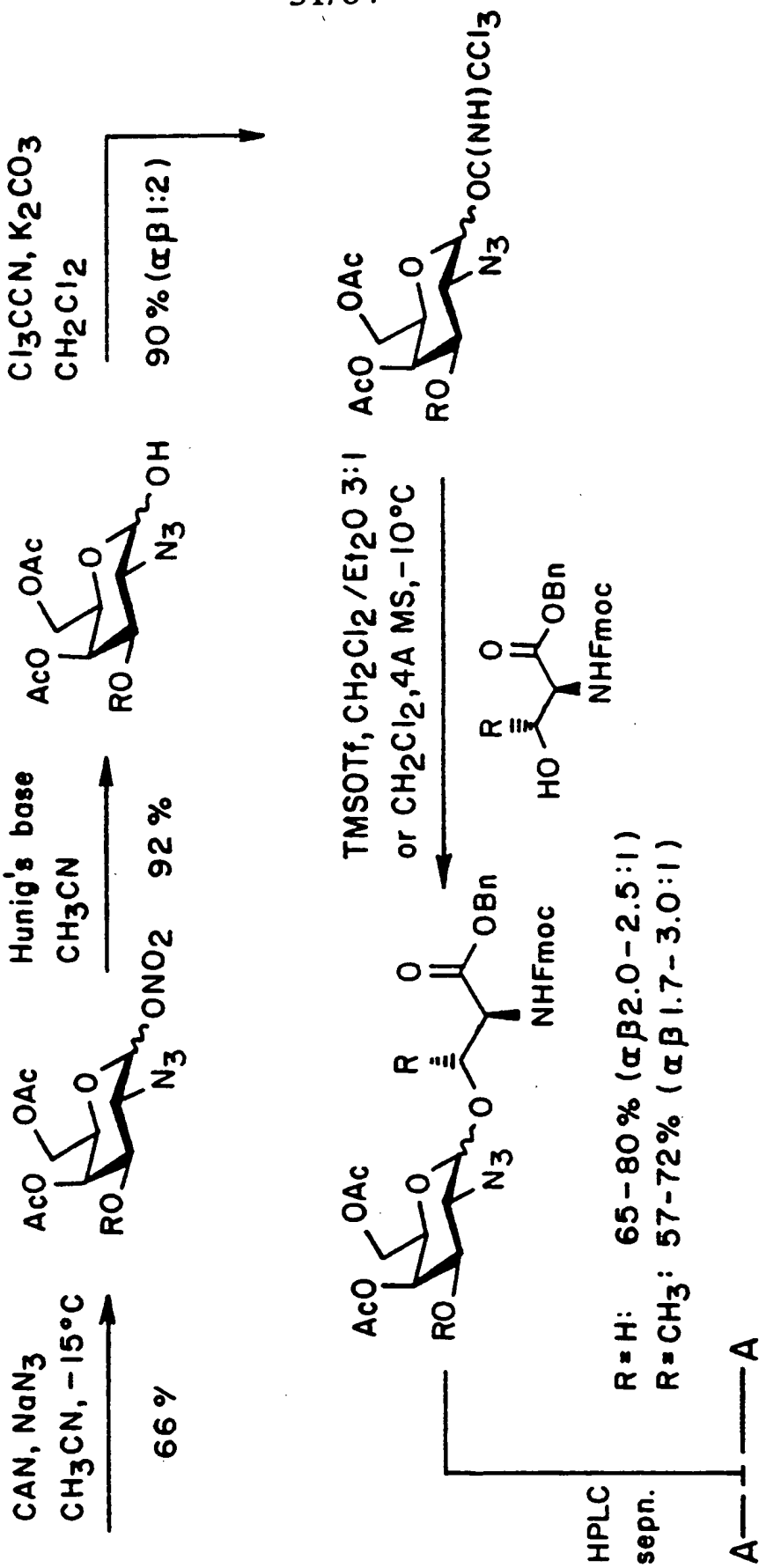

CAN, NaN3
CH3CN, -15°C
66 %
thiophenol
Hunig's base
CH3CN
92 %
Cl3CCN, K2CO3
CH2Cl2
90 % (αβ 1:2)
TMSOTf, CH2Cl2/Et2O 3:1
or CH2Cl2, 4A MS, -10°C
R = H: 65-80 % (αβ 2.0-2.5:1)
R = CH3: 57-72 % (αβ 1.7-3.0:1)
HPLC sepn.
A
A
AcO
RO
OAc
N3
ONO2
OH
OC(NH)CCl3
NHFmoc
OBn
HO

FIG. 17C

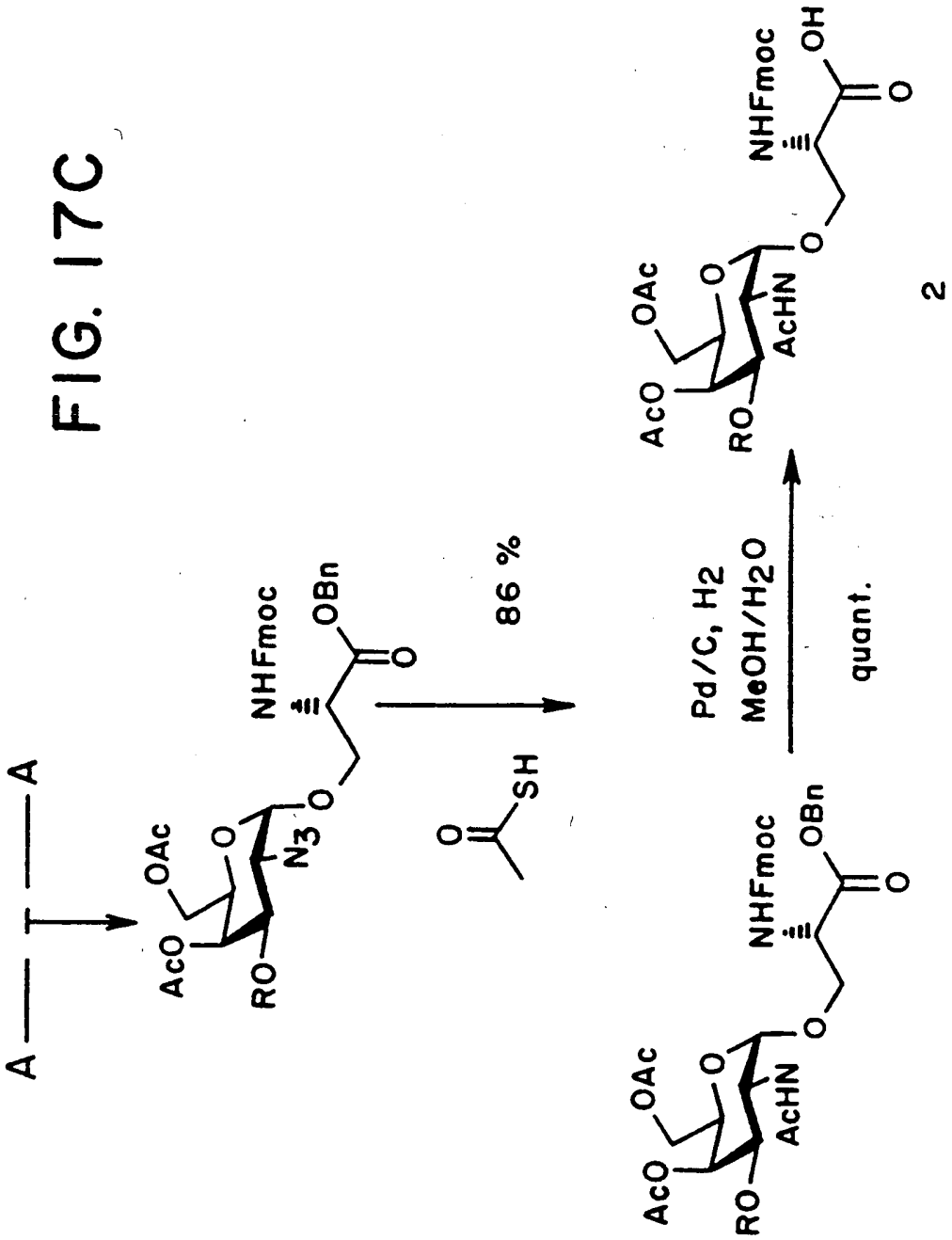
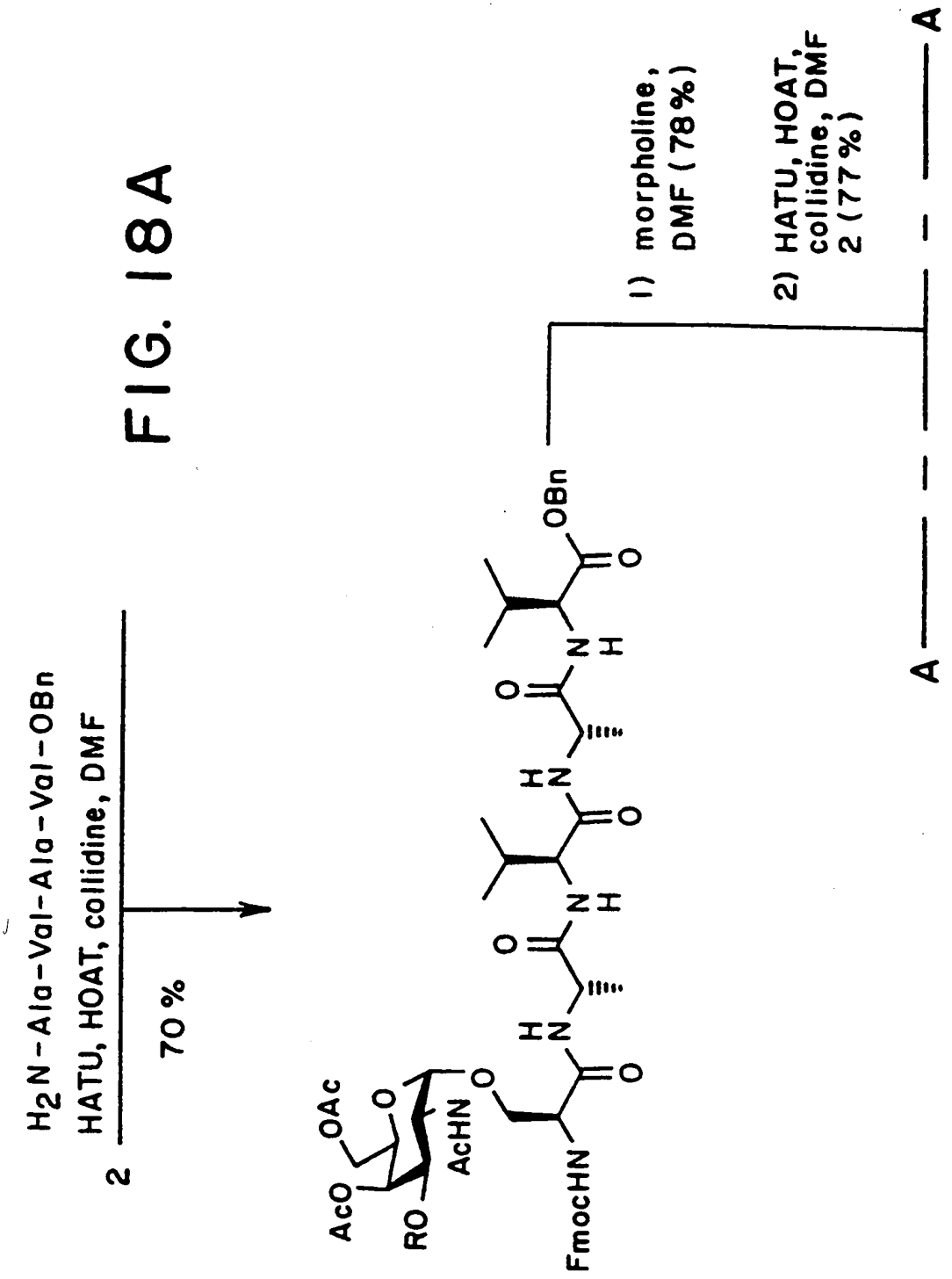

A
T
A
AcO
OAc
RO
N3
NHFmoc
OBn
86 %
SH
Pd/C, H2
MeOH/H2O
quant.
AcHN
OH
2

FIG. 18A


H2N-Ala-Val-Ala-Val-OBn
HATU, HOAT, collidine, DMF
70 %
2
1) morpholine, DMF (78%)
2) HATU, HOAT, collidine, DMF 2 (77%)
A
OBn
AcO
OAc
RO
AcHN
FmocHN

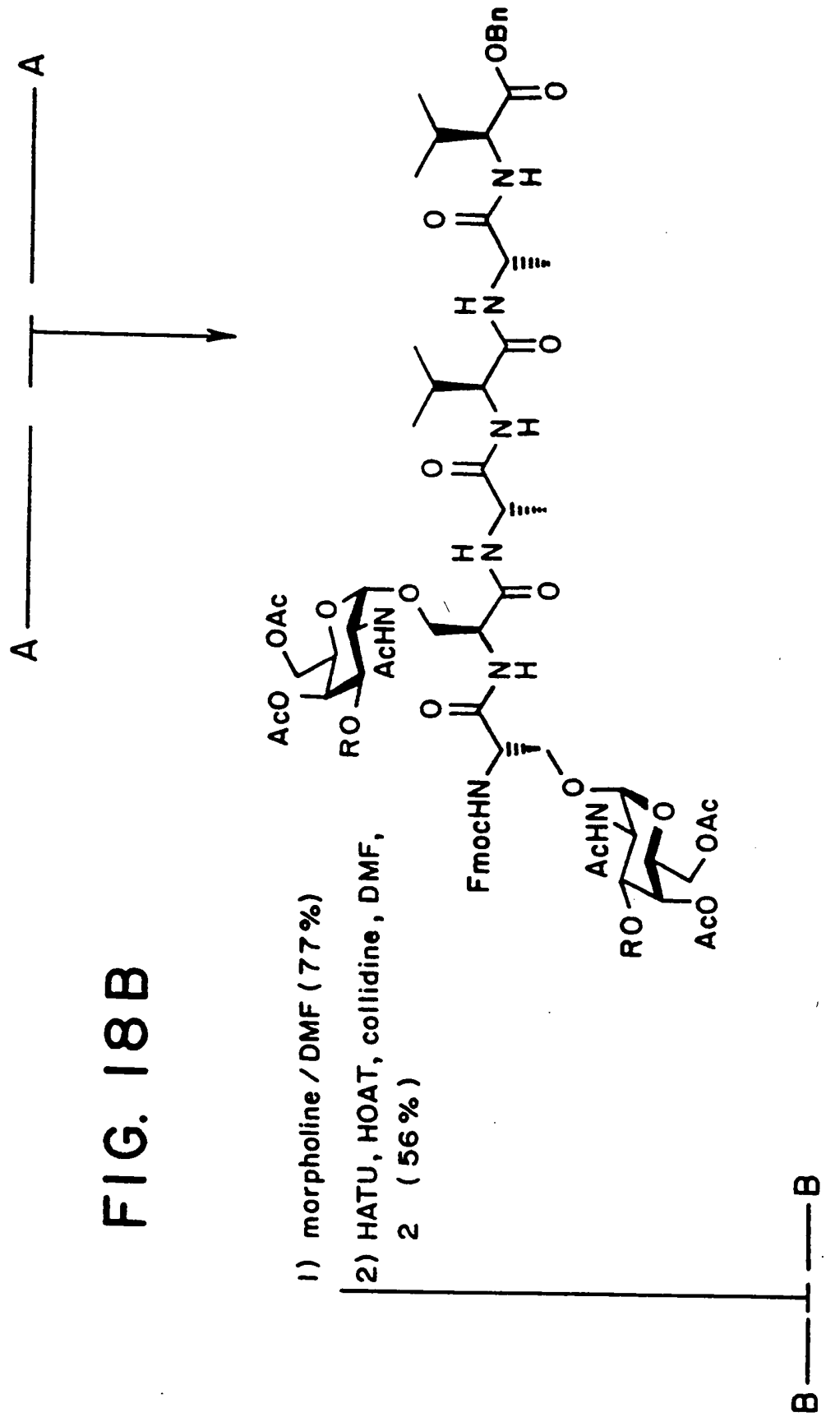

A
A
OBn
O
N
H
O
N
H
O
N
H
O
N
H
O
N
H
O
OAc
AcO
O
RO
AcHN
FmocHN
AcHN
O
OAc
RO
AcO
1) morpholine / DMF (77%)
2) HATU, HOAT, collidine, DMF,
2 (56%)
B
B

FIG. 18B

FIG. 18C

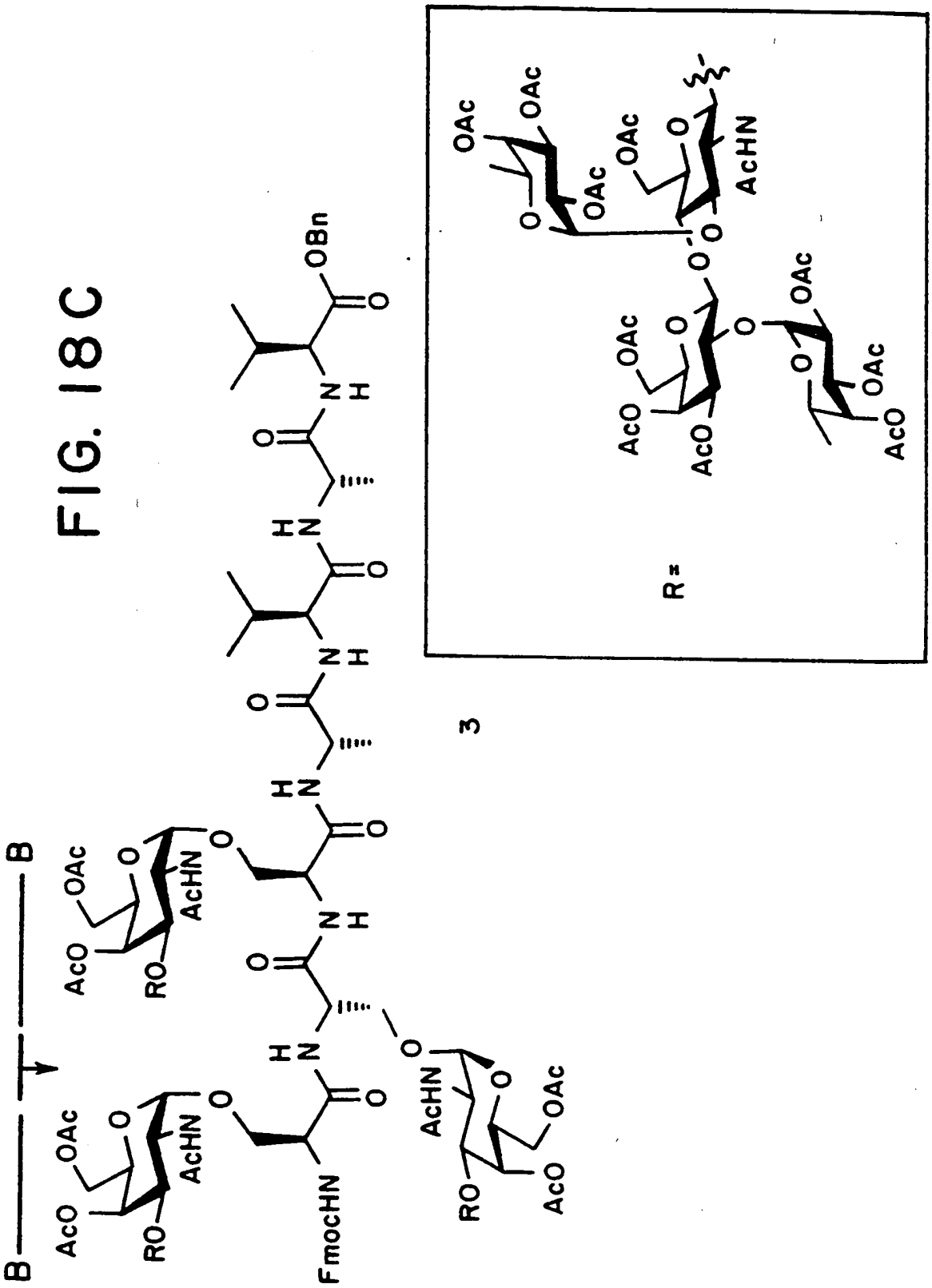


FIG. 19A

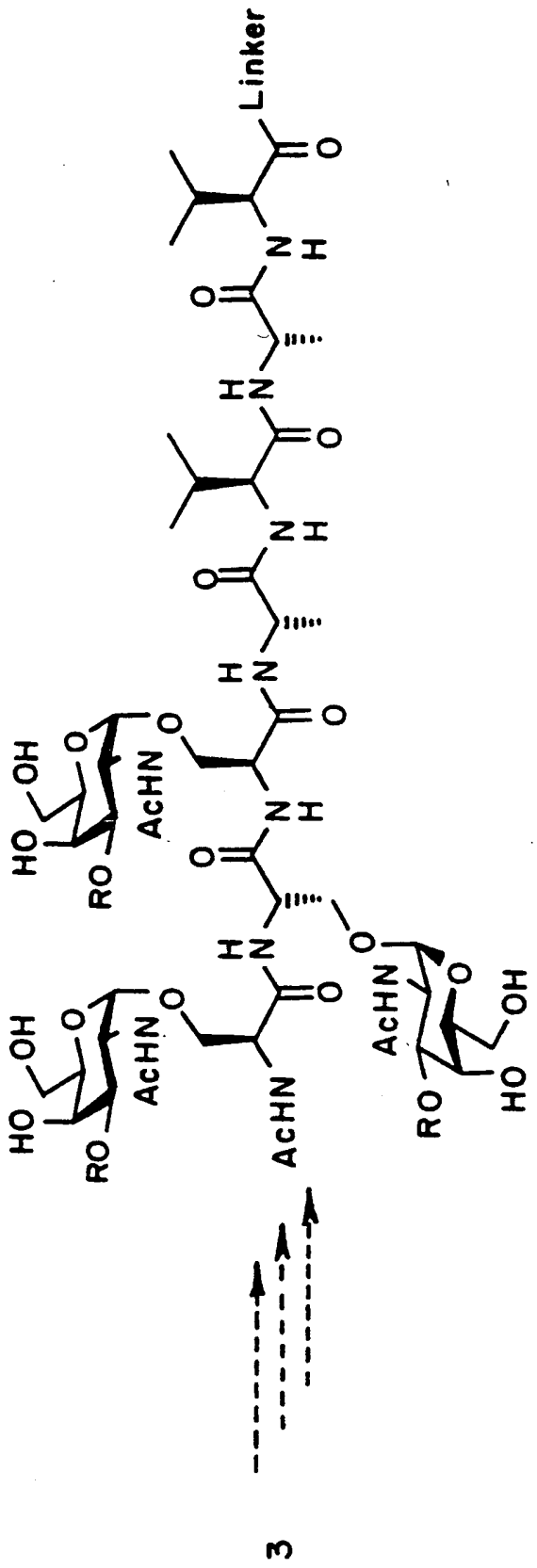

Linker
O
NH
O
HN
O
NH
O
HN
O
O
NH
O
HN
O
O
HO
OH
O
AcHN
RO
O
HO
OH
O
AcHN
RO
AcHN
O
AcHN
OH
O
RO
HO
3

FIG. 19B

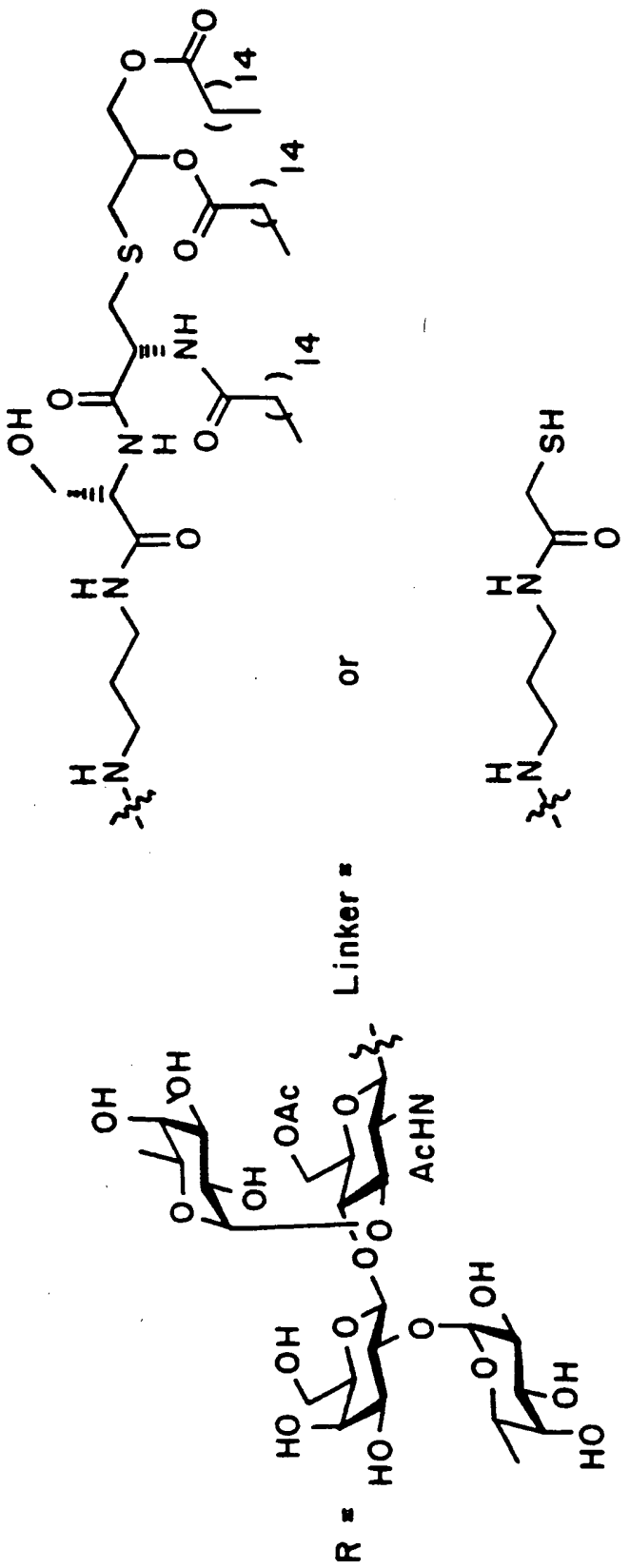


FIG. 20A

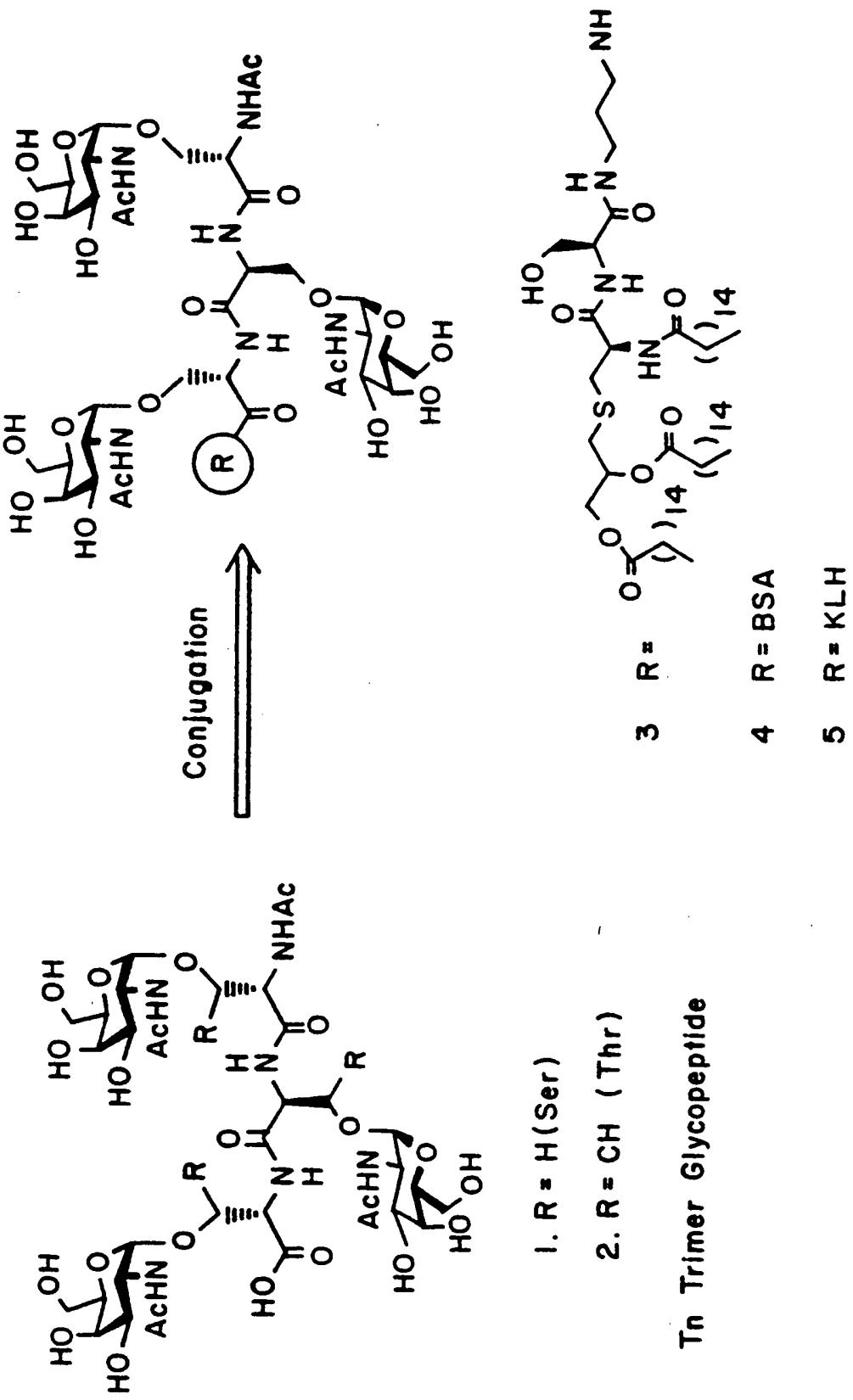

Conjugation
1. R = H(Ser)
2. R = CH (Thr)
Tn Trimer Glycopeptide
3 R=
4 R= BSA
5 R= KLH

FIG. 20B

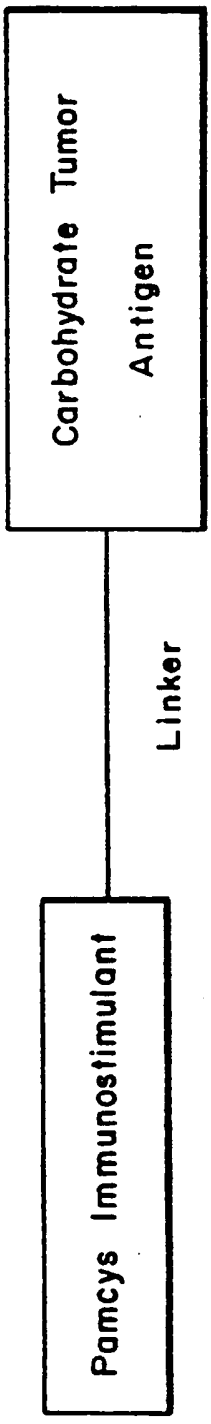
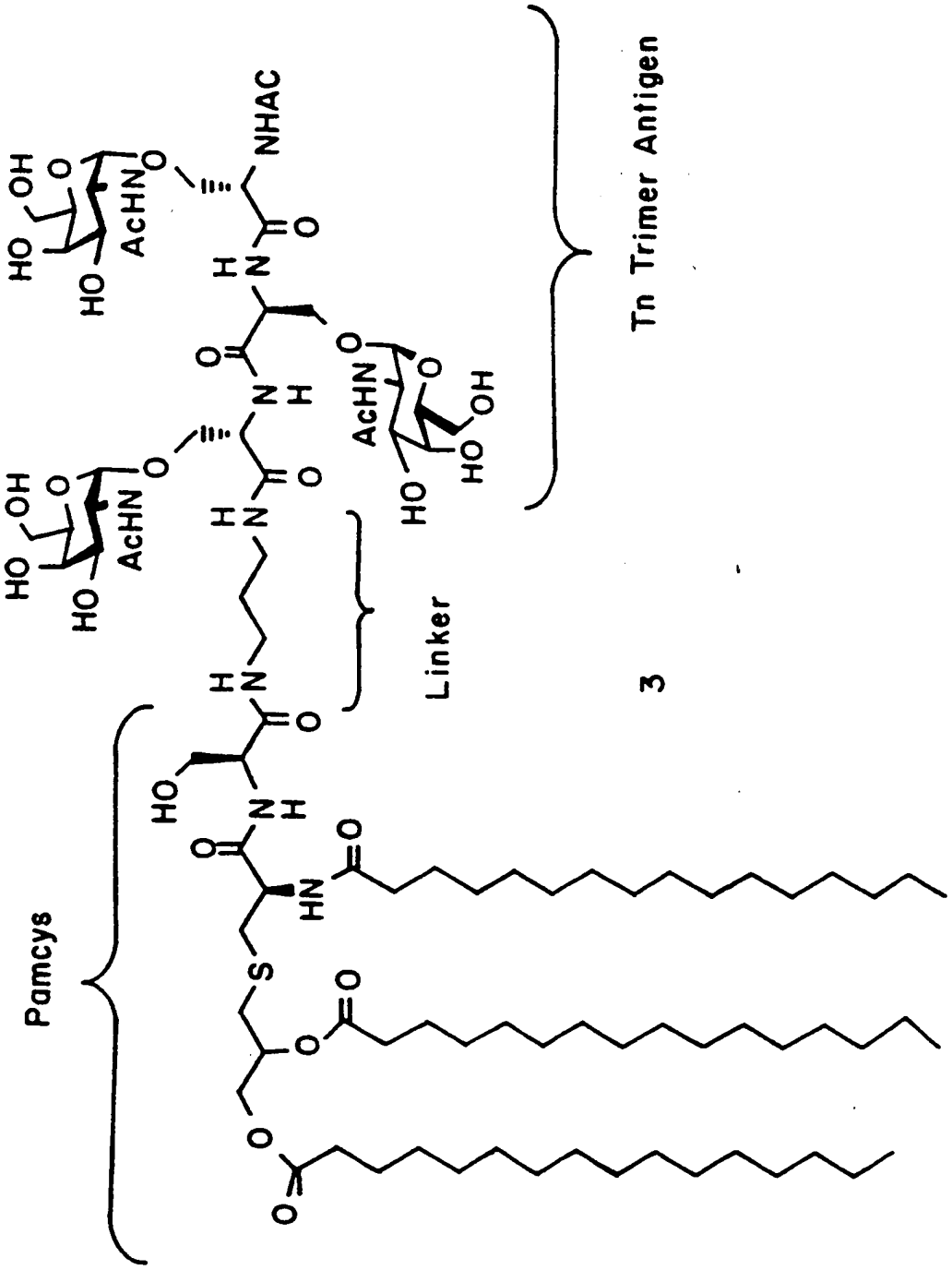

Carbohydrate Tumor Antigen
Linker
Pamcys Immunostimulant

FIG. 20C



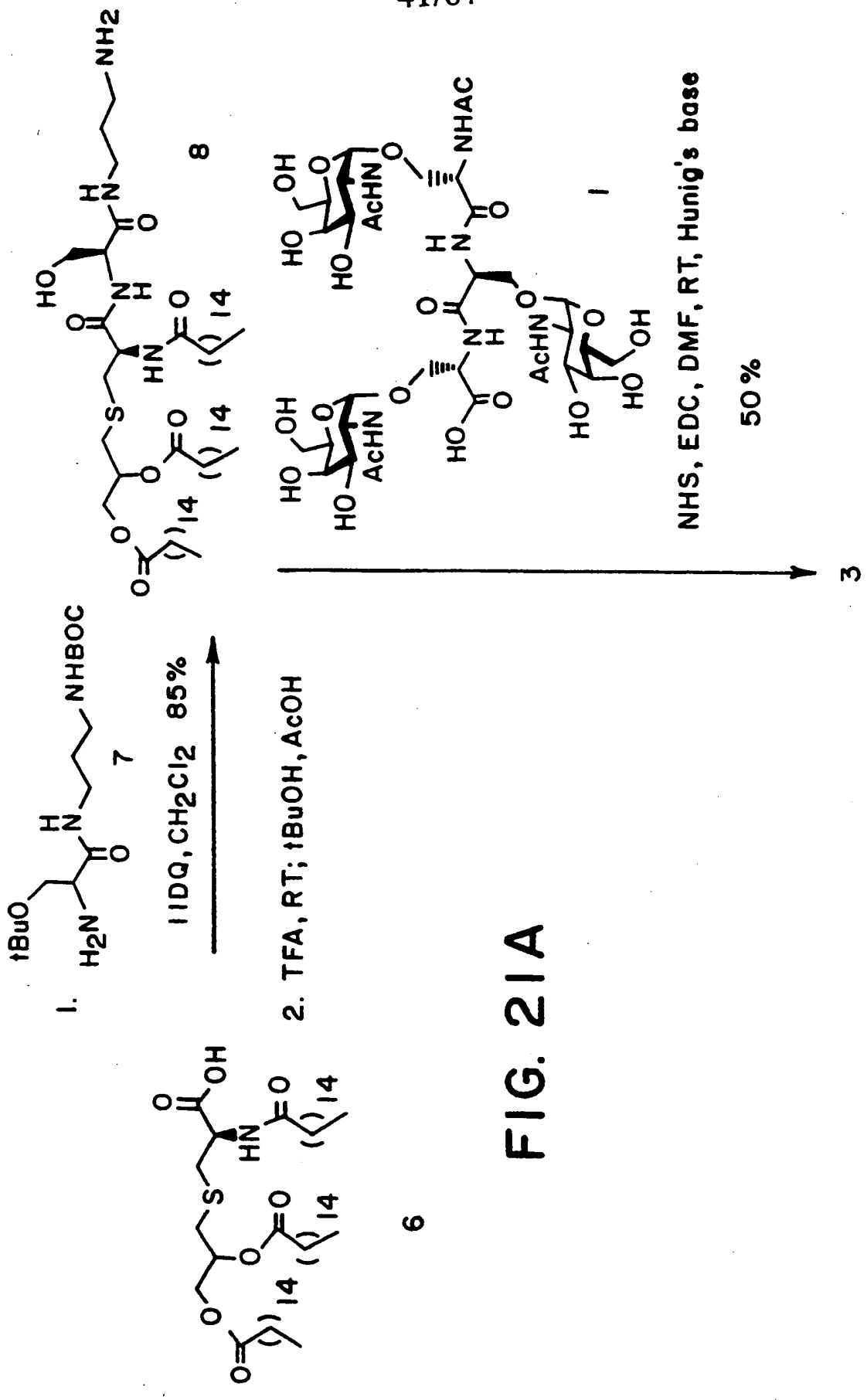
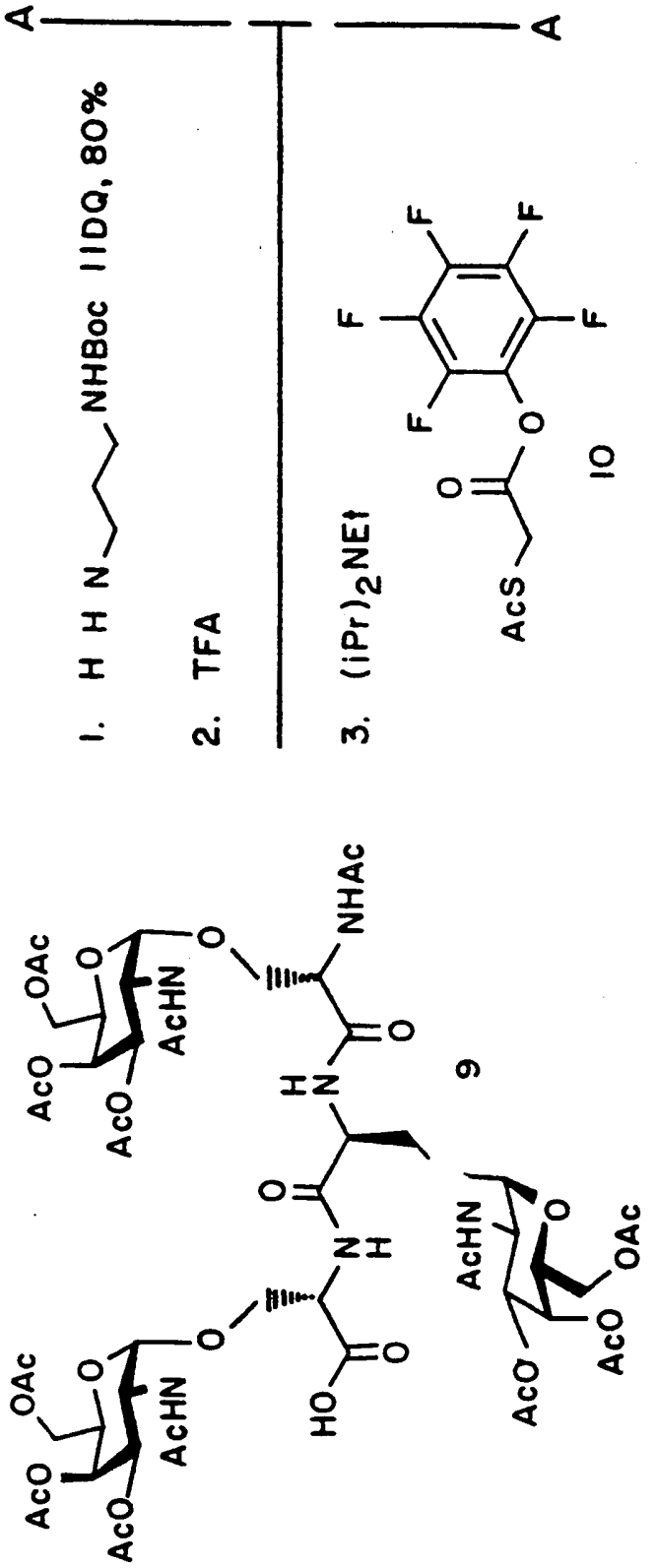

1.
2. TFA, RT; tBuOH, AcOH
IIDQ, CH2Cl2 85%
NHS, EDC, DMF, RT, Hunig's base
50 %
3

FIG. 21A

FIG. 21B


A
A
1. H H N NHBoc IIDQ, 80%
2. TFA
3. (iPr)2NEt
10
4. NaOMe, MeOH
under Argon, degased MeOH
70% for 3 steps
9
AcO
OAc
AcHN
NHAc
HO

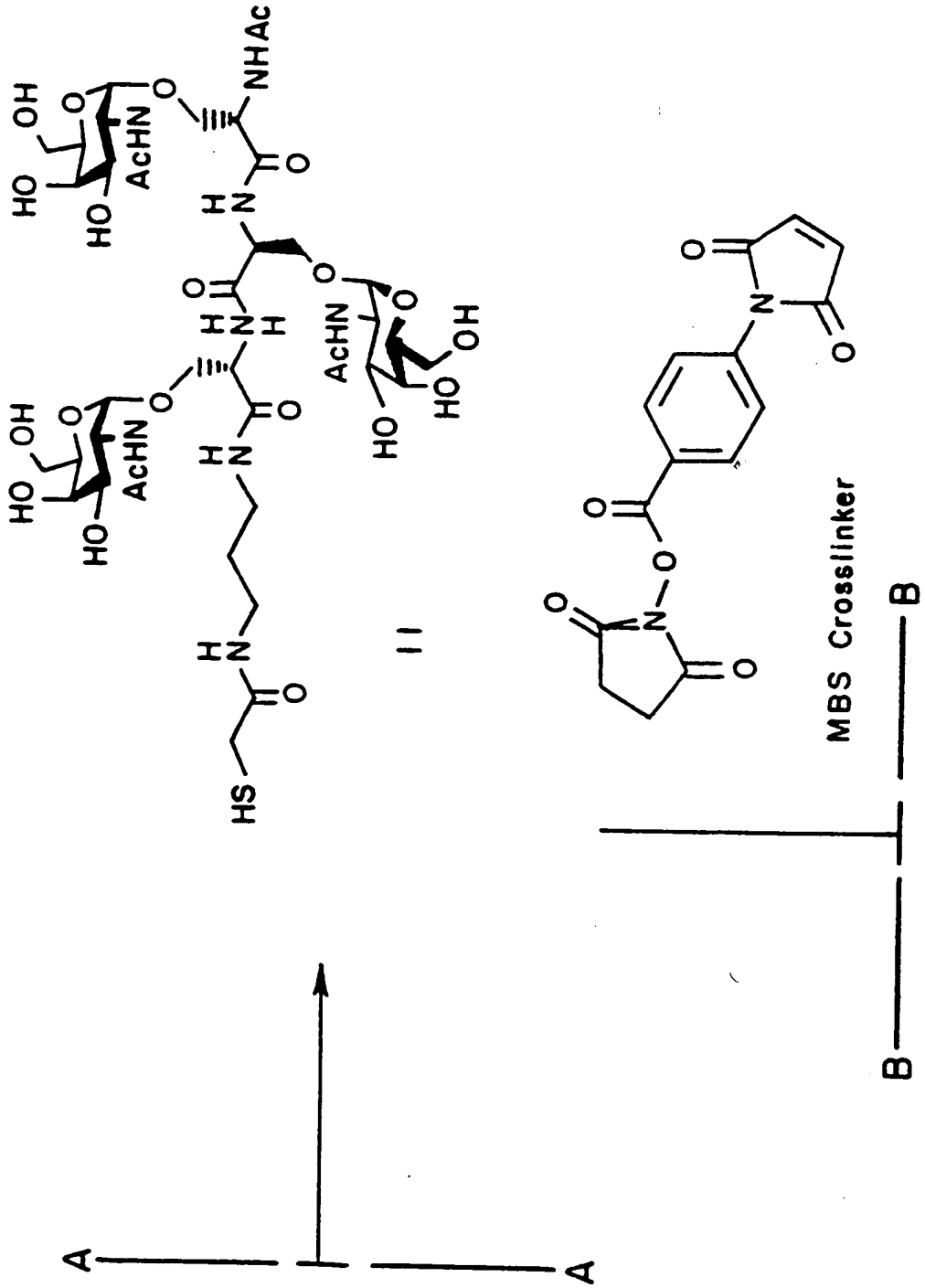


FIG. 21C

FIG. 21D

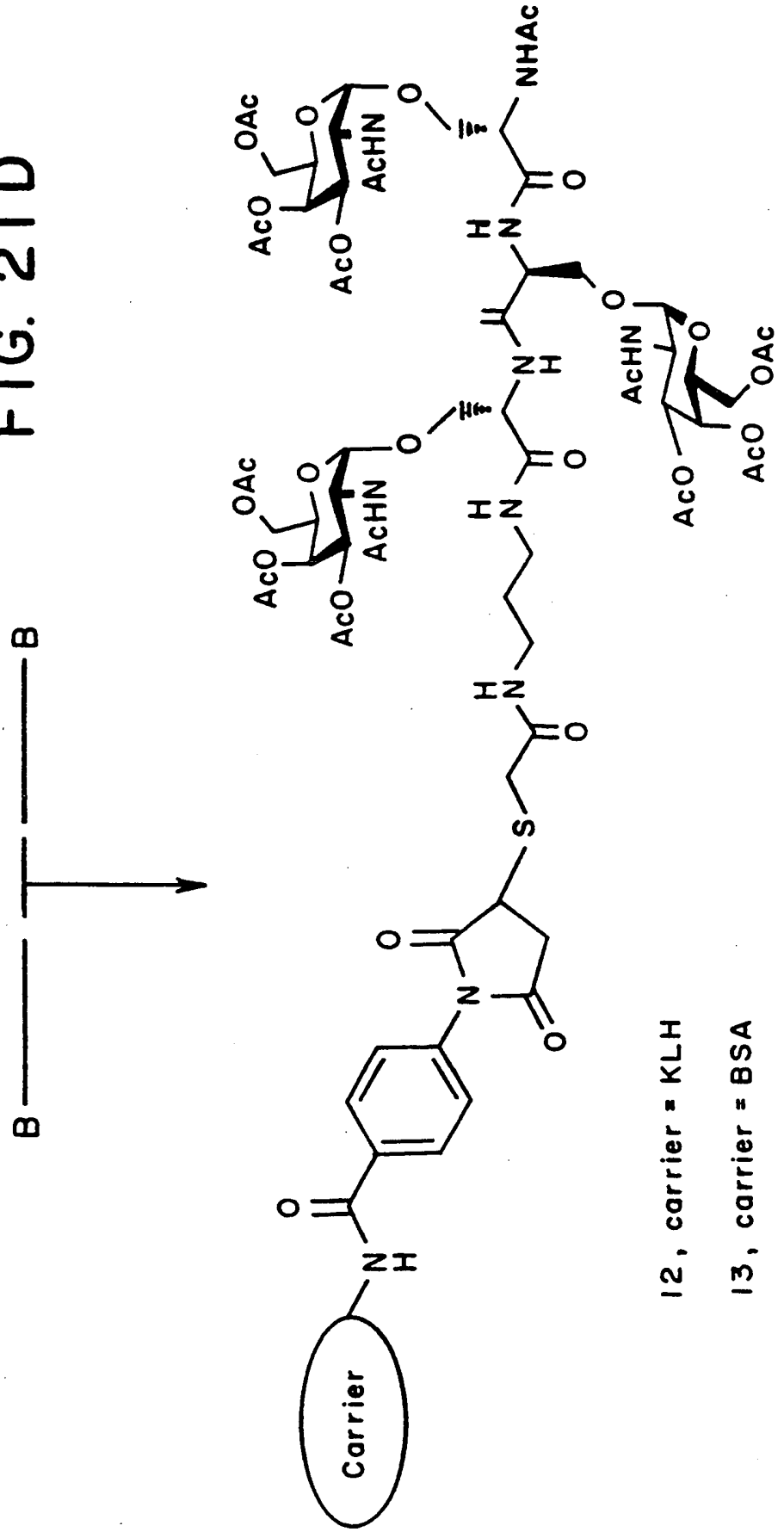


FIG. 22A

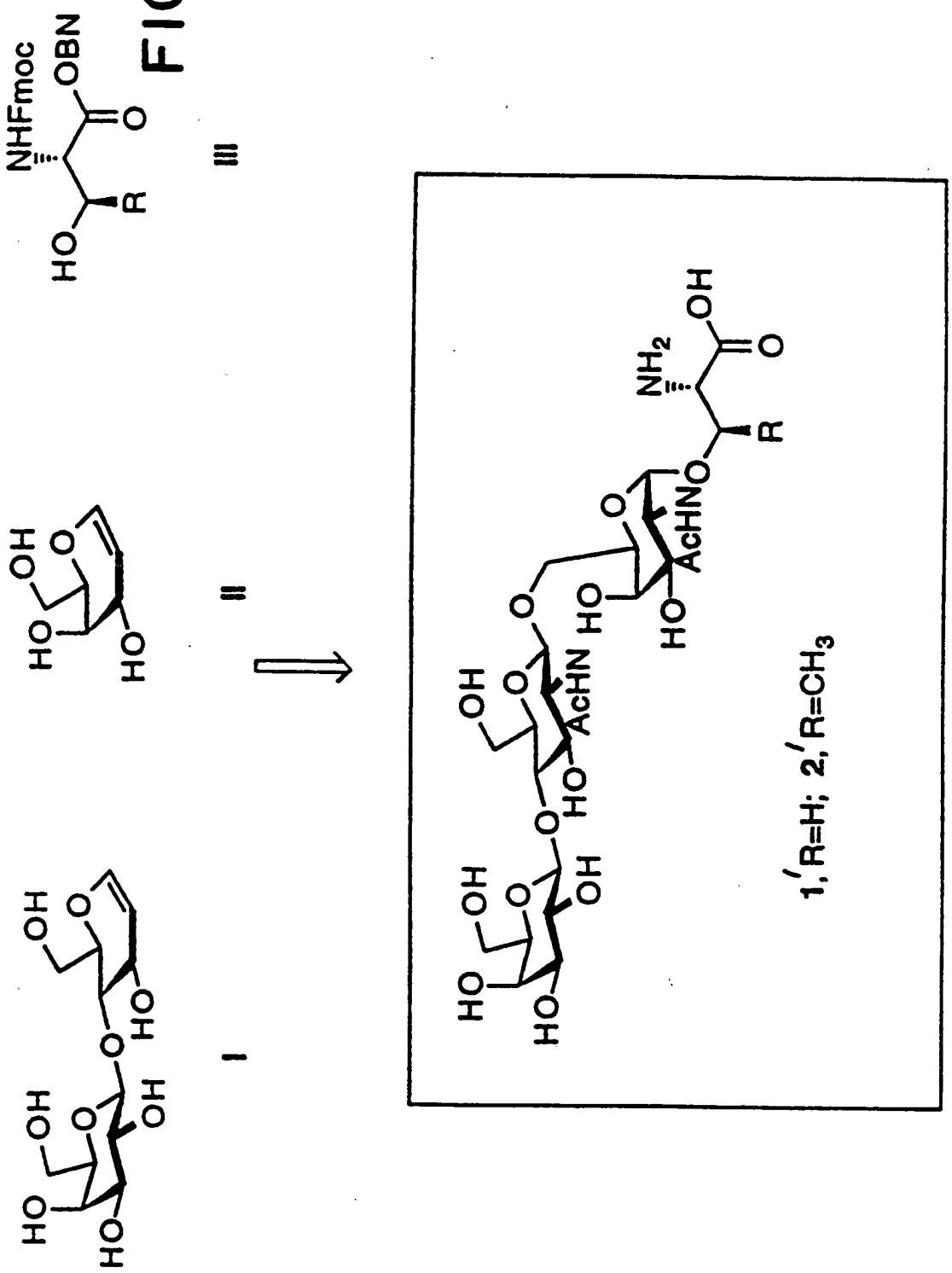
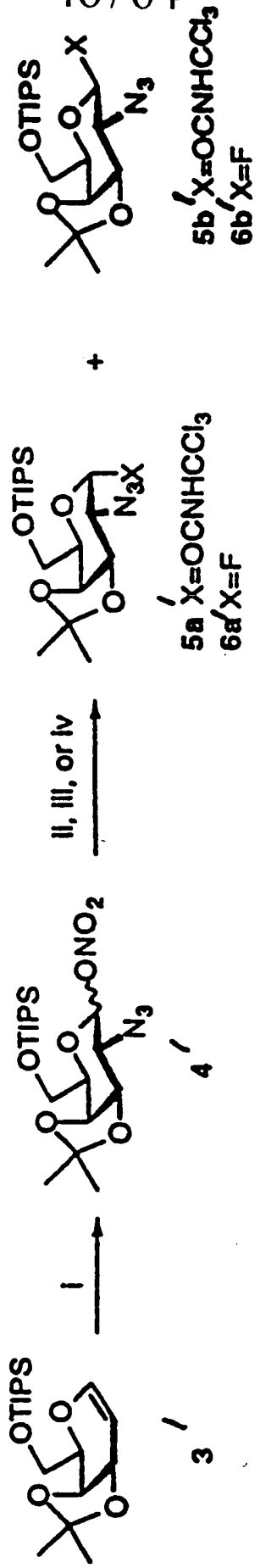


FIG. 22B


OTIPS
O
O
O
3'
i
OTIPS
O
O
ONO2
N3
4'
II, III, or IV
OTIPS
O
O
N3X
5a' X=OCNHCCl3
6a' X=F
+
OTIPS
X
O
N3
O
O
5b' X=OCNHCCl3
6b' X=F

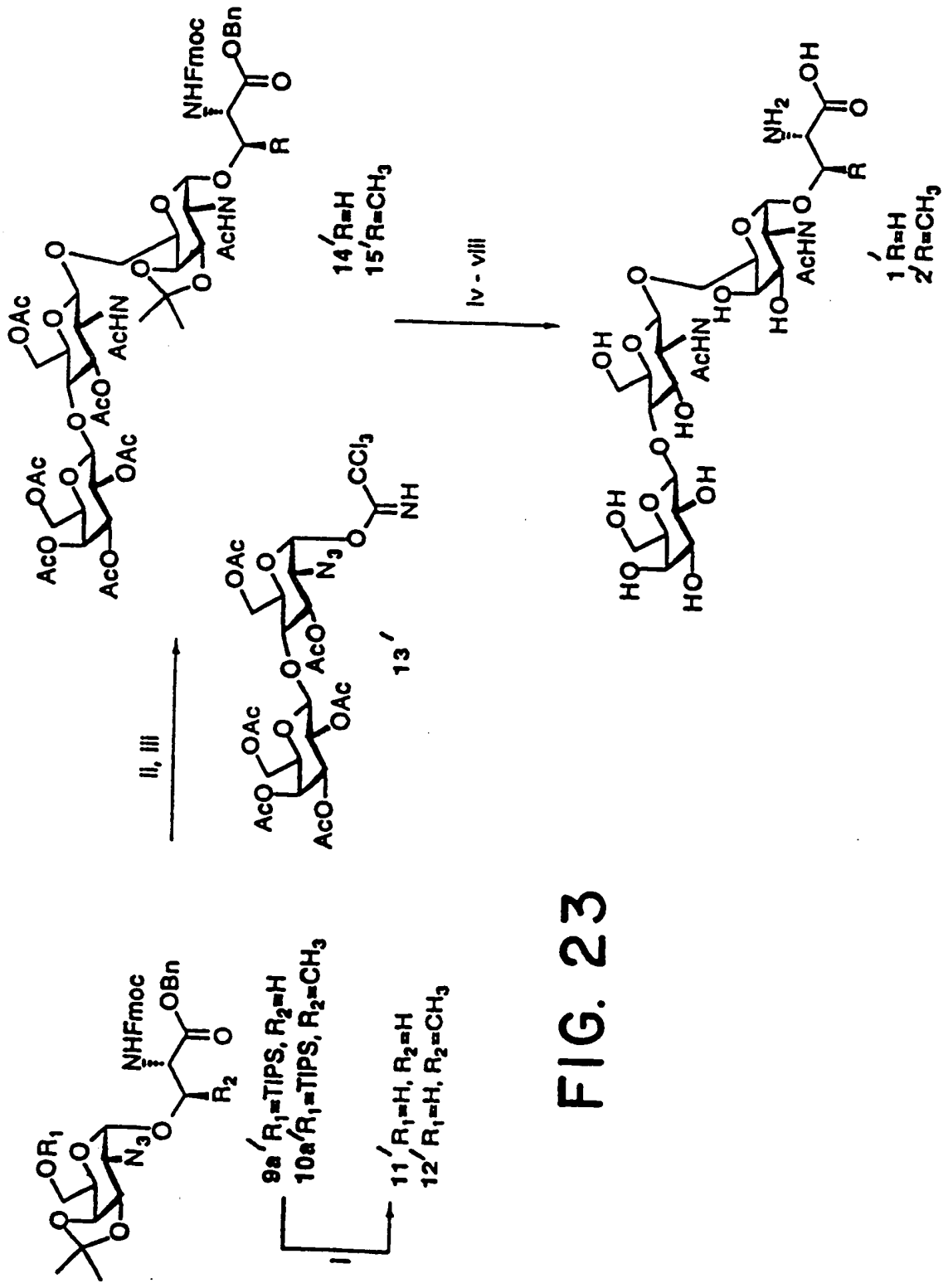

NHFmoc
OBn
AcHN
AcO
OAc
OR1
N3
R2
9a' R1=TIPS, R2=H
10a' R1=TIPS, R2=CH3
11' R1=H, R2=H
12' R1=H, R2=CH3
I
II, III
13'
CCl3
NH
14' R=H
15' R=CH3
IV - VIII
NH2
OH
HO
1' R=H
2' R=CH3

FIG. 23

FIG. 24

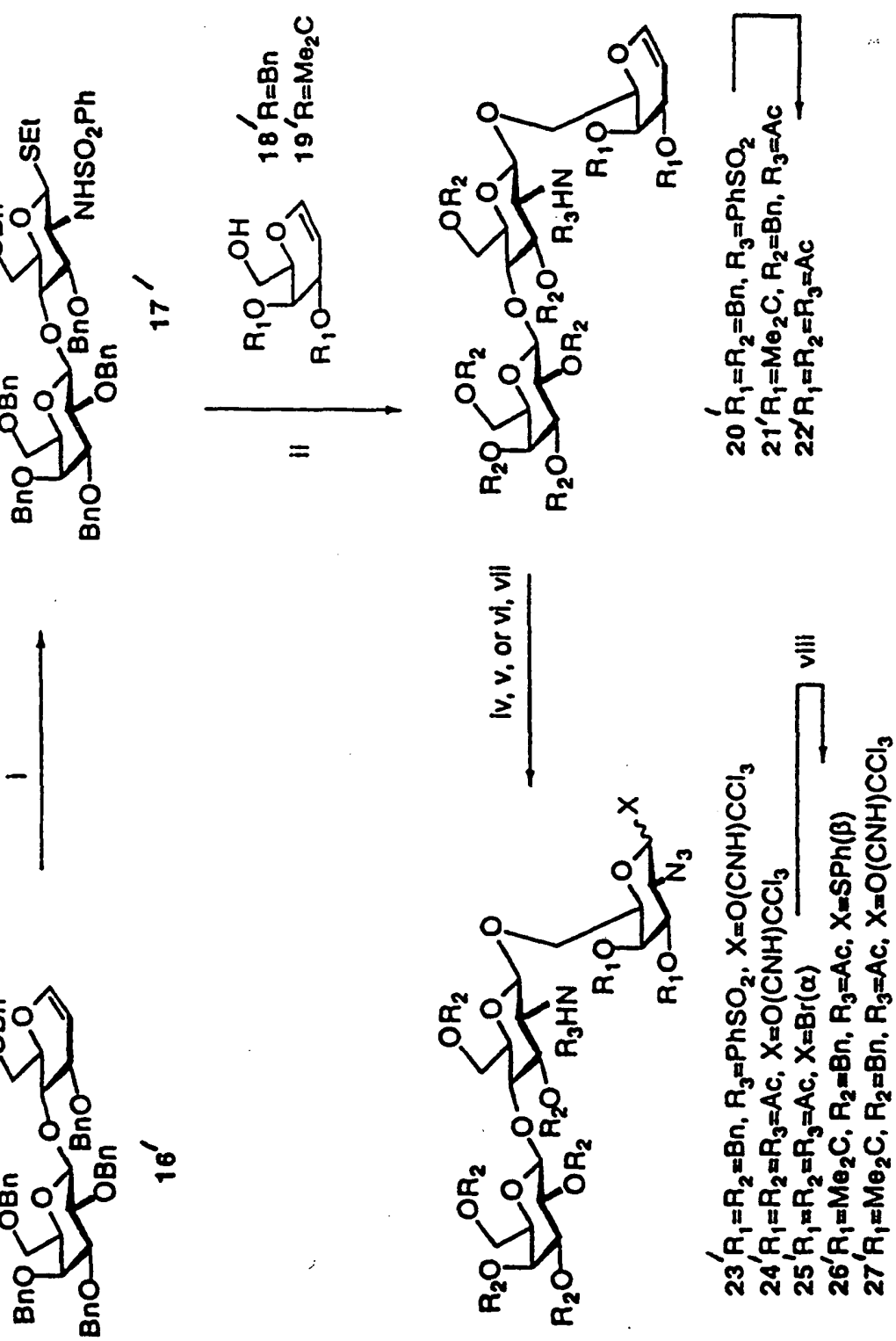

16′
17′
18′ R=Bn
19′ R=Me2C
20′ R1=R2=Bn, R3=PhSO2
21′ R1=Me2C, R2=Bn, R3=Ac
22′ R1=R2=R3=Ac
23′ R1=R2=Bn, R3=PhSO2, X=O(CNH)CCl3
24′ R1=R2=R3=Ac, X=O(CNH)CCl3
25′ R1=R2=R3=Ac, X=Br(α)
26′ R1=Me2C, R2=Bn, R3=Ac, X=SPh(β)
27′ R1=Me2C, R2=Bn, R3=Ac, X=O(CNH)CCl3
i
ii
iii
iv, v, or vi, vii
viii

FIG. 25A

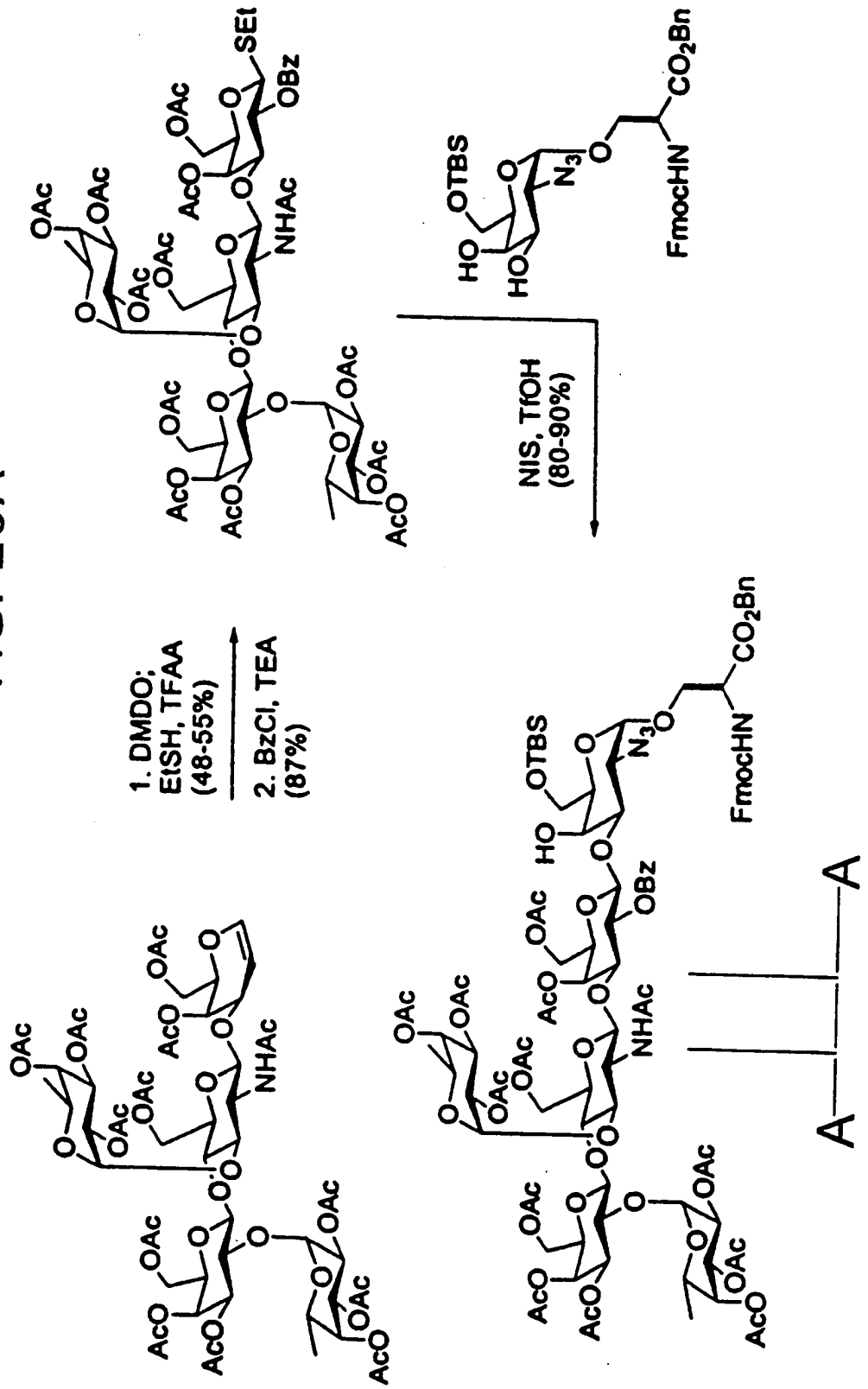


FIG. 25B

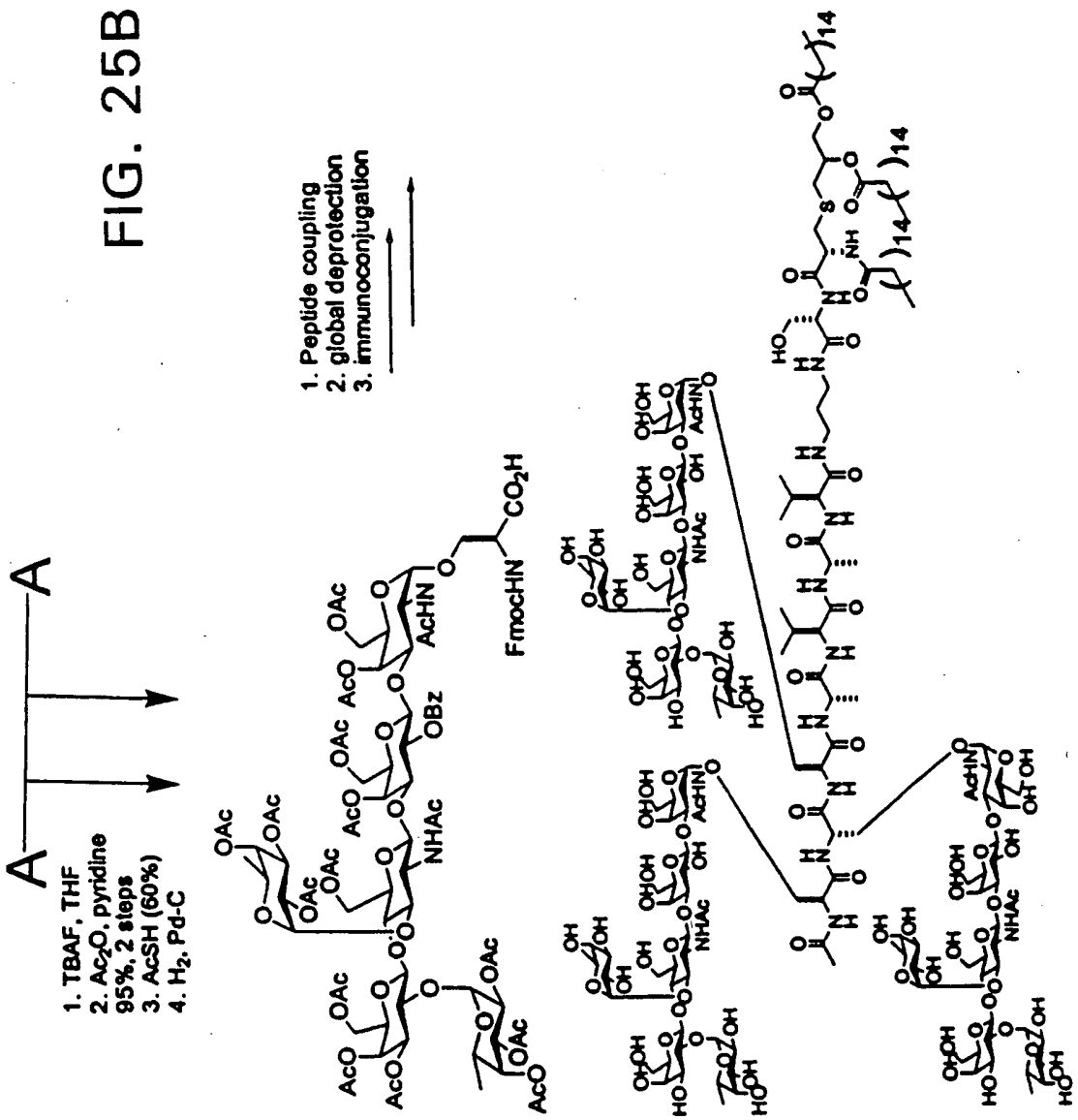
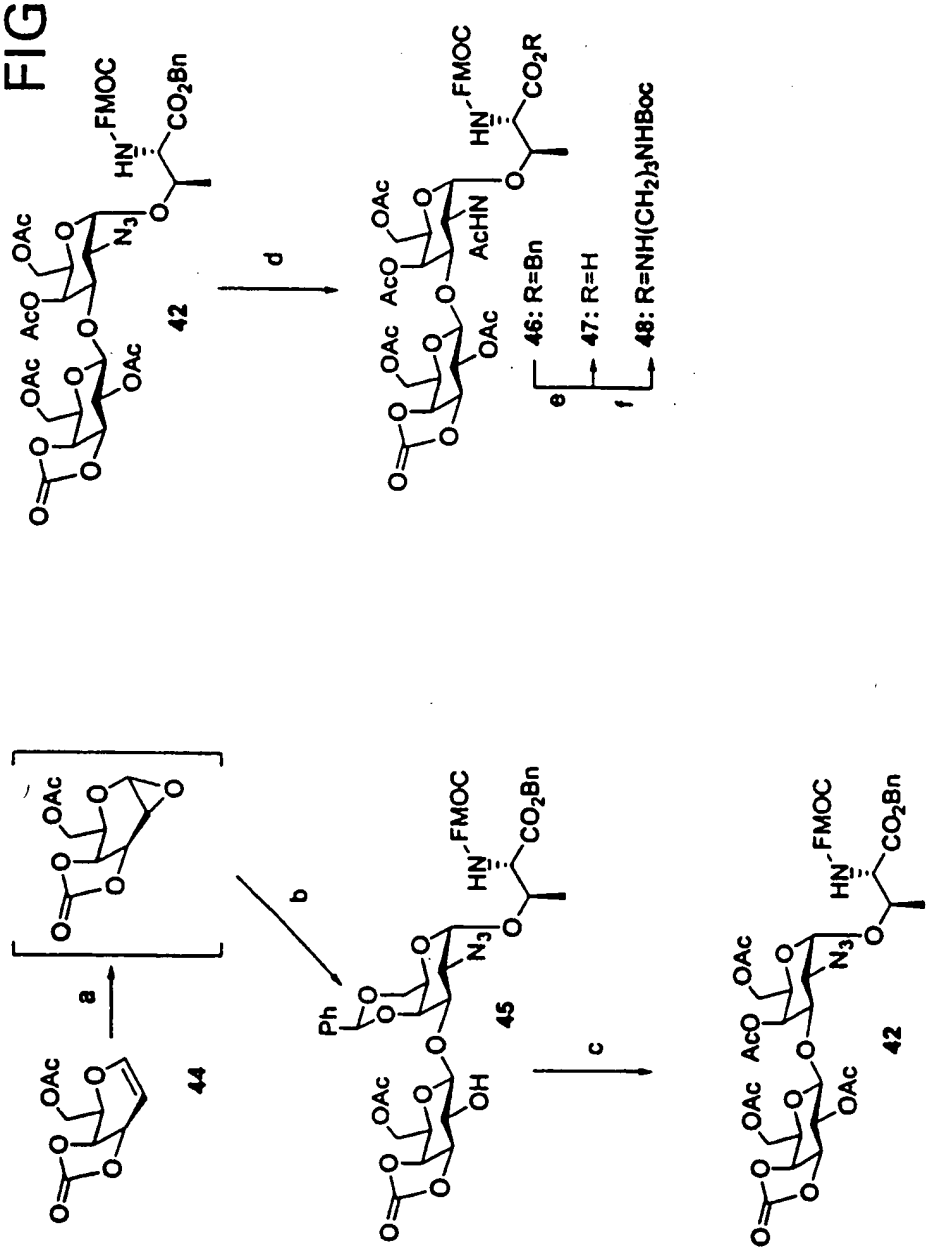

A
A
1. TBAF, THF
2. Ac2O, pyridine
95%, 2 steps
3. AcSH (60%)
4. H2, Pd-C
1. Peptide coupling
2. global deprotection
3. immunoconjugation
FmocHN
CO2H
AcHN
NHAc
OBz
OAc
AcO
HO
OH
Z
14

FIG. 26



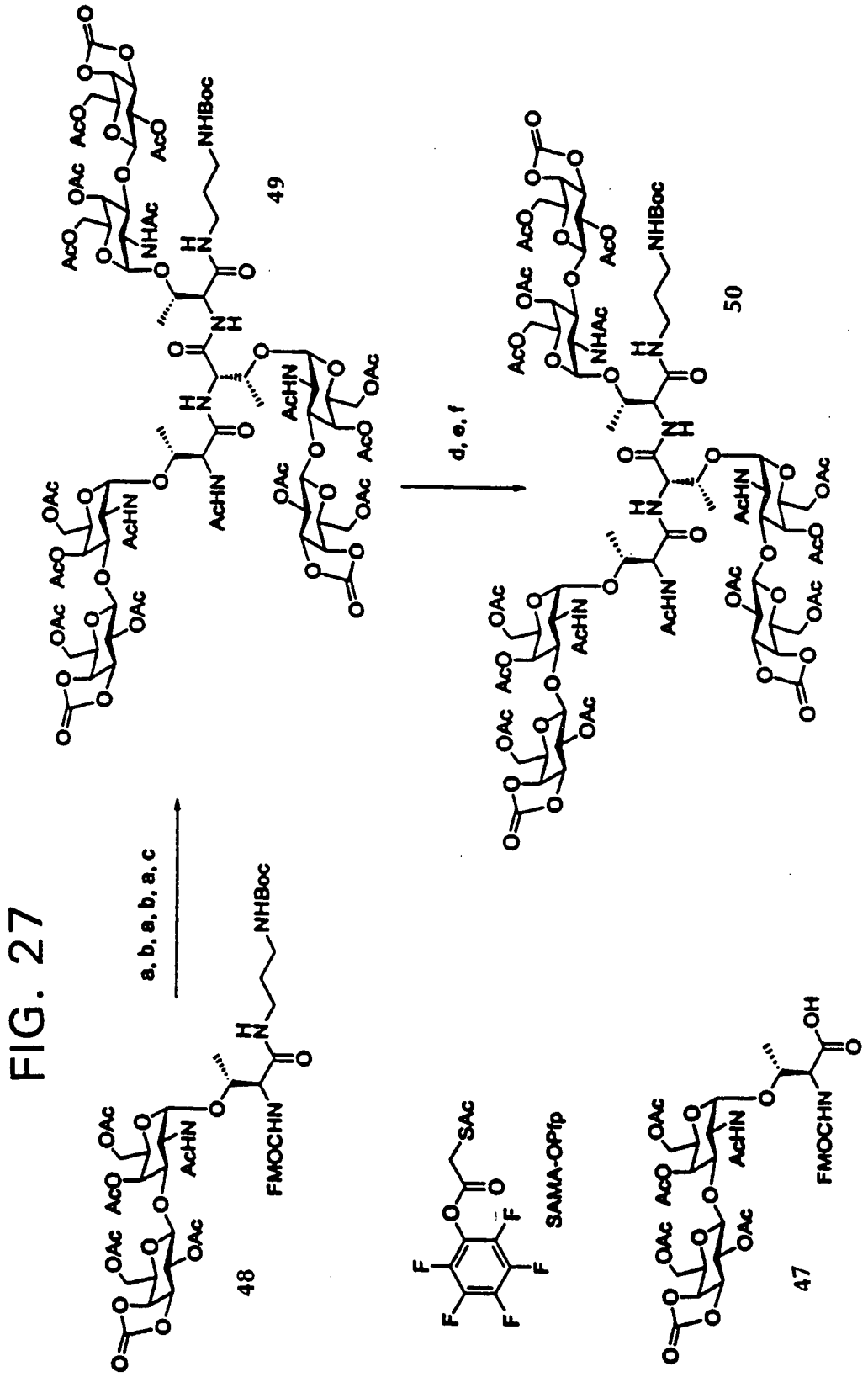

a, b, a, b, a, c
d, e, f
48
49
50
47
SAMA-OPfp
FMOCHN
NHBoc
AcHN
NHAc
AcO
OAc
SAc
OH
F

FIG. 27

FIG. 28A

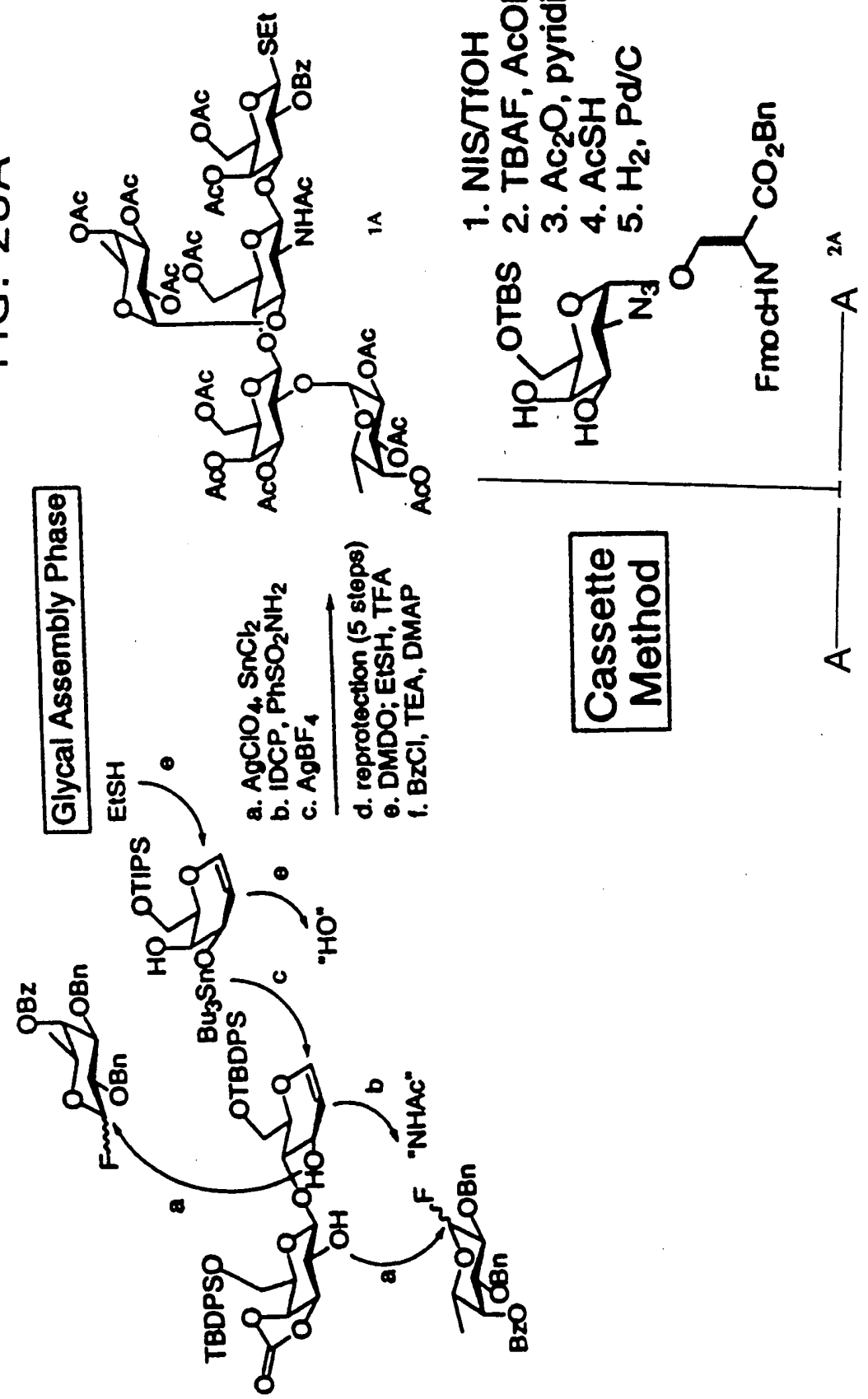

Glycal Assembly Phase
EtSH
OTIPS
HO
Bu3SnO
OTBDPS
TBDPSO
OBz
OBn
F
OH
"HO"
"NHAc"
a. AgClO4, SnCl2
b. IDCP, PhSO2NH2
c. AgBF4
d. reprotection (5 steps)
e. DMDO; EtSH, TFA
f. BzCl, TEA, DMAP
1A
1. NIS/TfOH
2. TBAF, AcOH
3. Ac2O, pyridine
4. AcSH
5. H2, Pd/C
OTBS
N3
FmocHN
CO2Bn
2A
Cassette Method
A

FIG. 28B

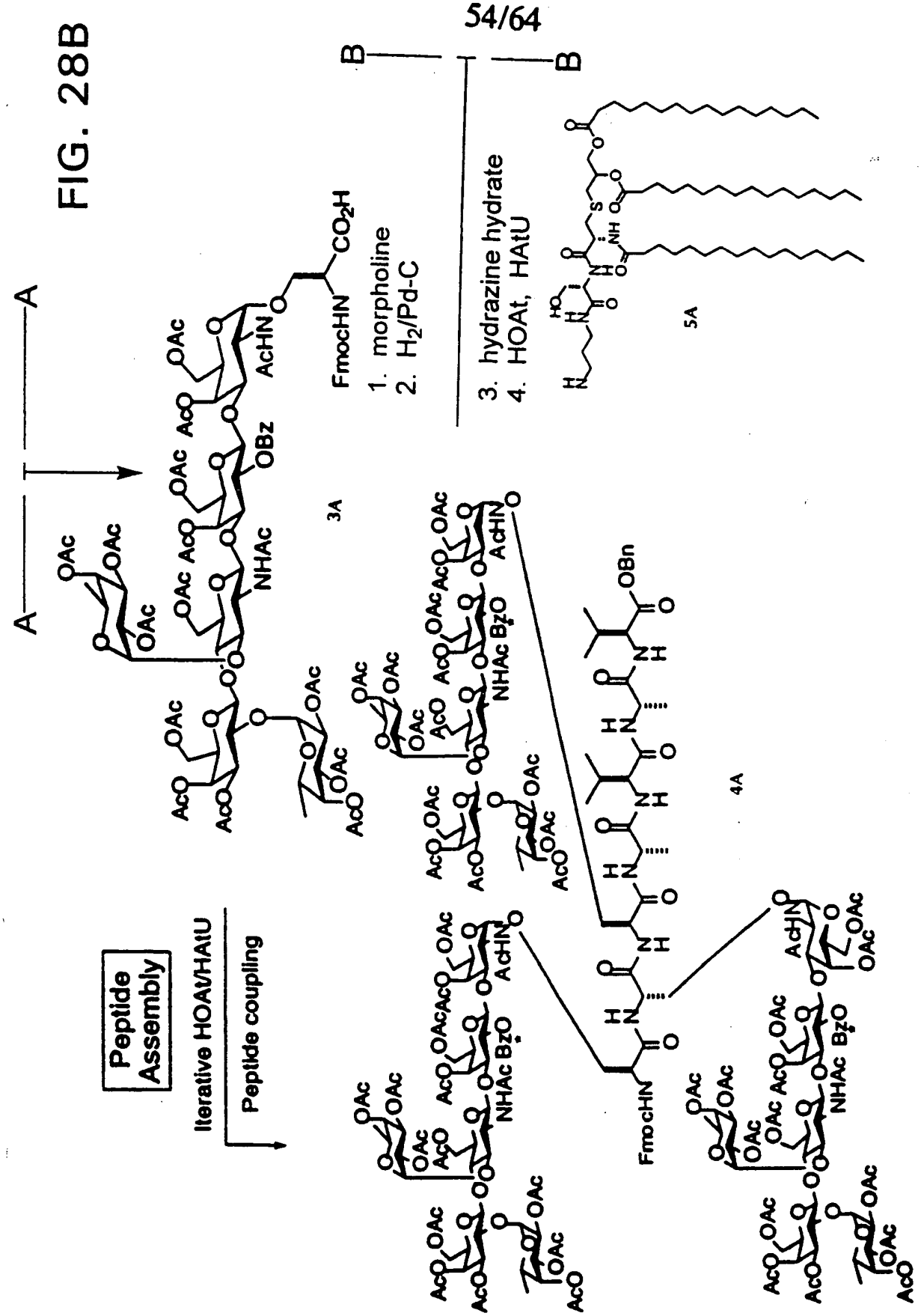

A
A
B
B
1. morpholine
2. H_2/Pd-C
3. hydrazine hydrate
4. HOAt, HAtU
3A
4A
5A
Peptide Assembly
Iterative HOAt/HAtU
Peptide coupling

FIG. 28C

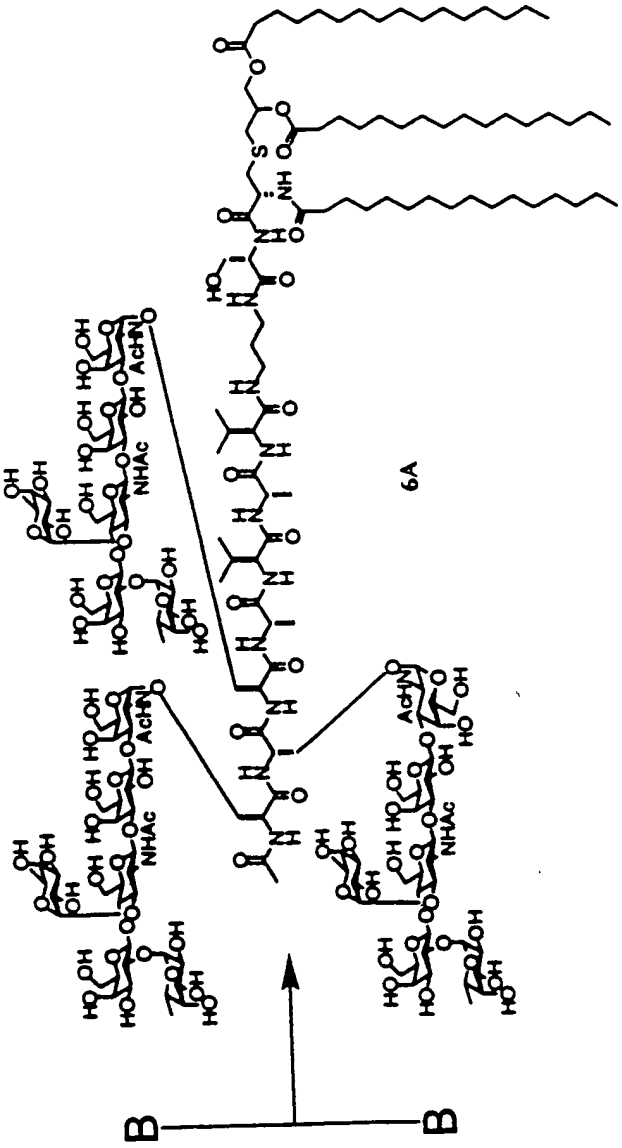
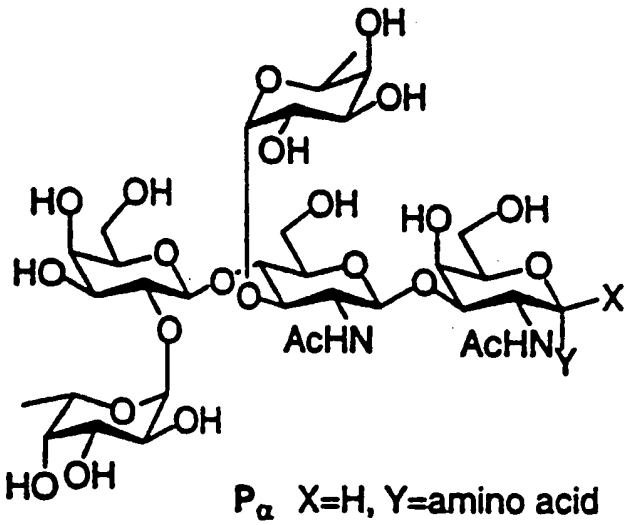


FIG. 29A



P_α X=H, Y=amino acid

P_β X=amino acid, Y=H

FIG. 29B

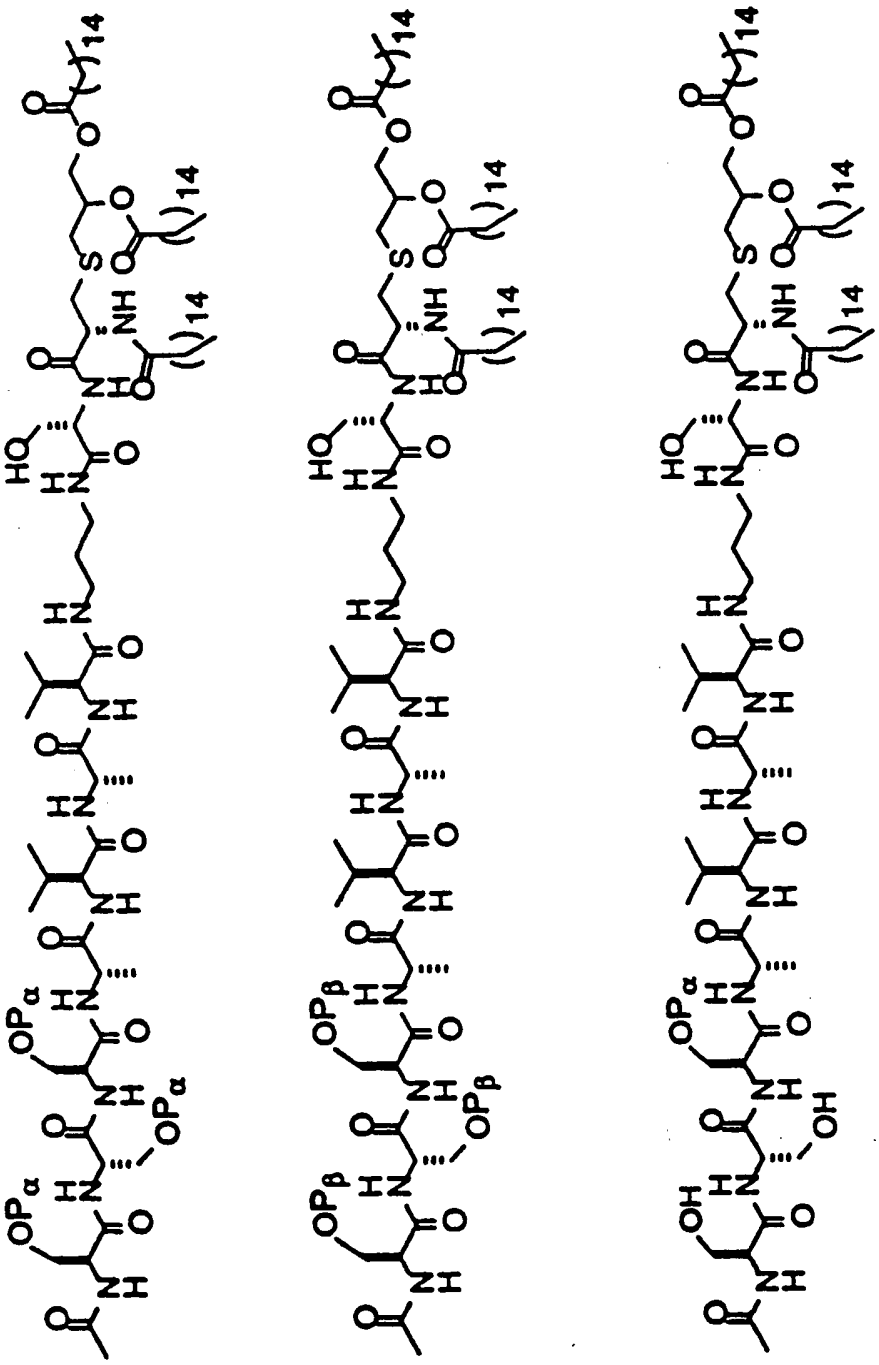


FIG. 30

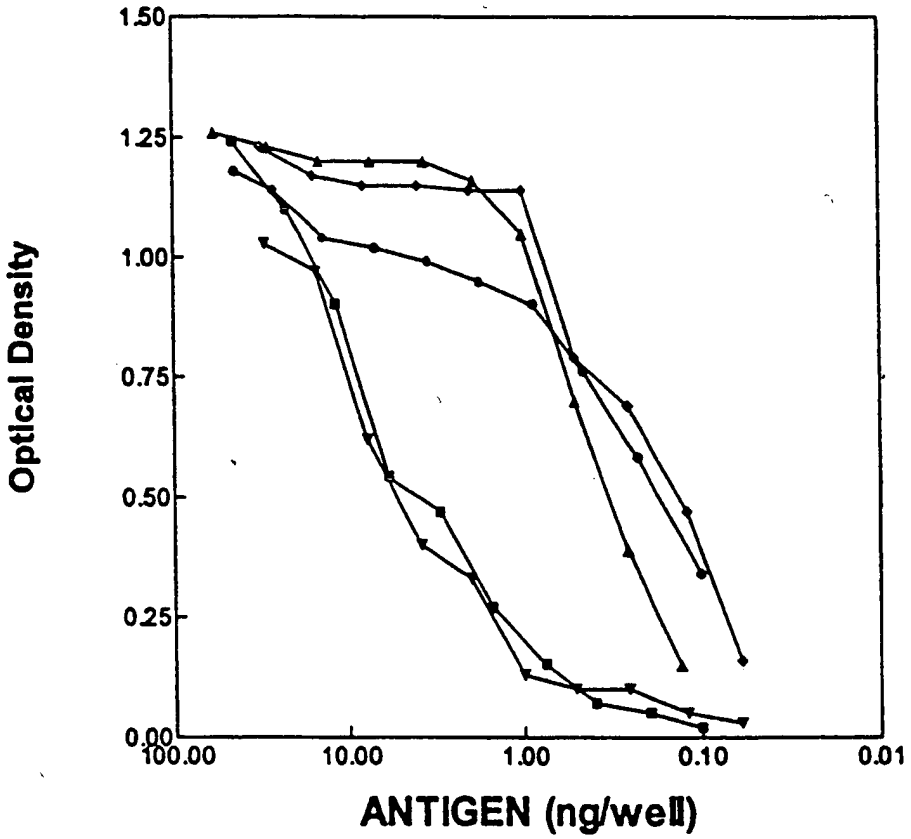

1.50
1.25
1.00
0.75
0.50
0.25
0.00
Optical Density
100.00
10.00
1.00
0.10
0.01
ANTIGEN (ng/well)

FIG. 31A

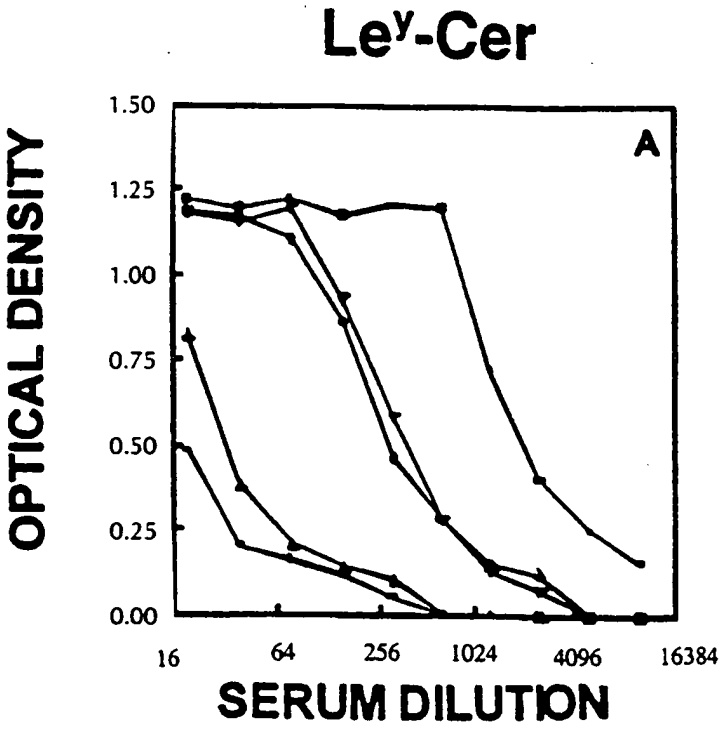

Le^y-Cer
A
OPTICAL DENSITY
1.50
1.25
1.00
0.75
0.50
0.25
0.00
16
64
256
1024
4096
16384
SERUM DILUTION

FIG. 31B

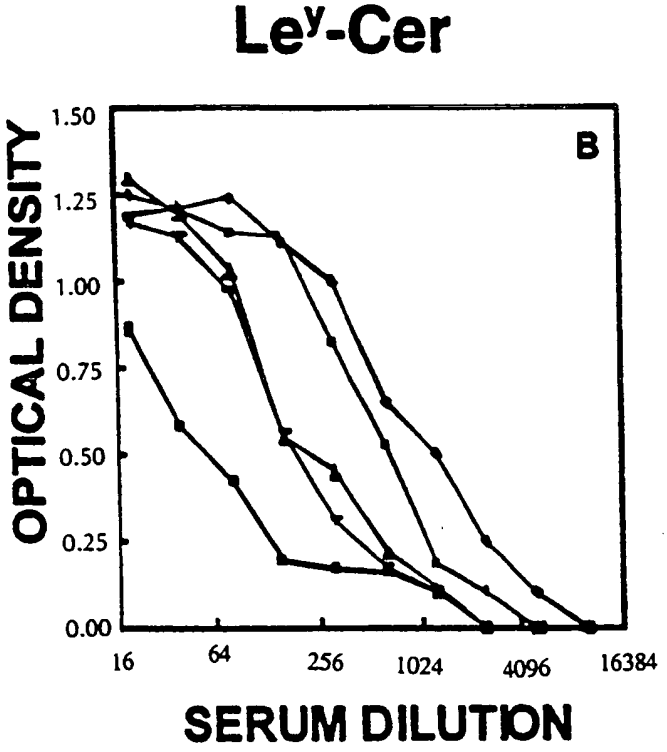

Le^y-Cer
B
OPTICAL DENSITY
1.50
1.25
1.00
0.75
0.50
0.25
0.00
16
64
256
1024
4096
16384
SERUM DILUTION

FIG. 31C

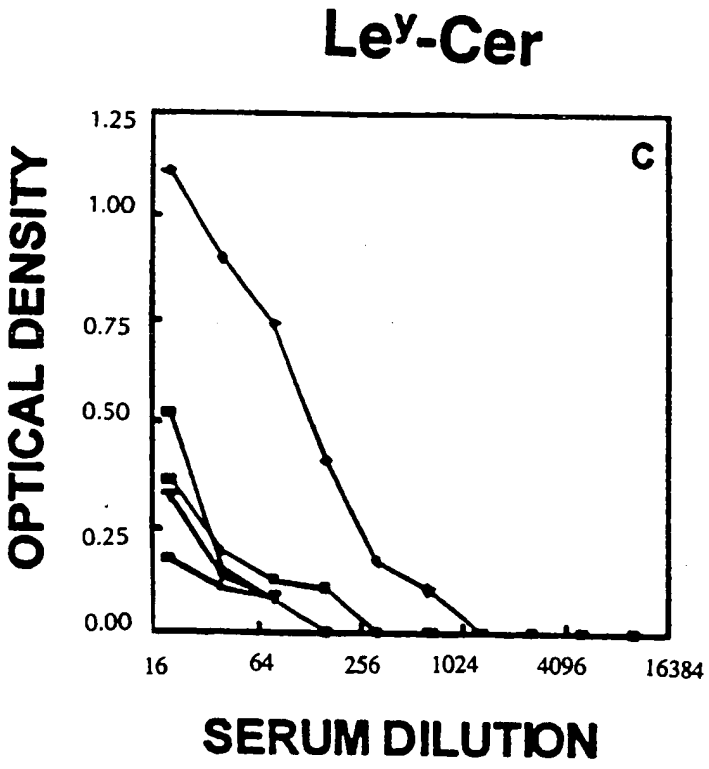

Le^y-Cer
C
OPTICAL DENSITY
1.25
1.00
0.75
0.50
0.25
0.00
16
64
256
1024
4096
16384
SERUM DILUTION

FIG. 31D

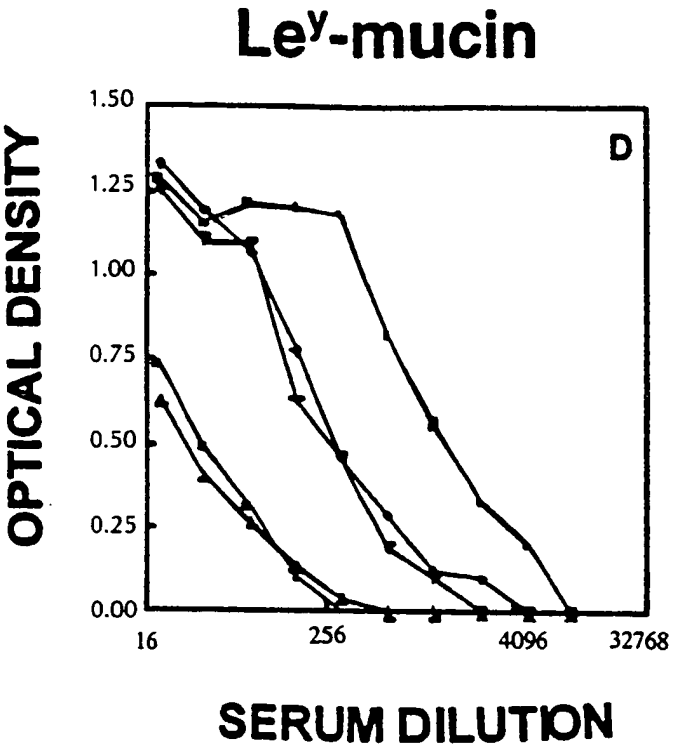

Le^y-mucin
D
OPTICAL DENSITY
1.50
1.25
1.00
0.75
0.50
0.25
0.00
16
256
4096
32768
SERUM DILUTION

FIG. 31E

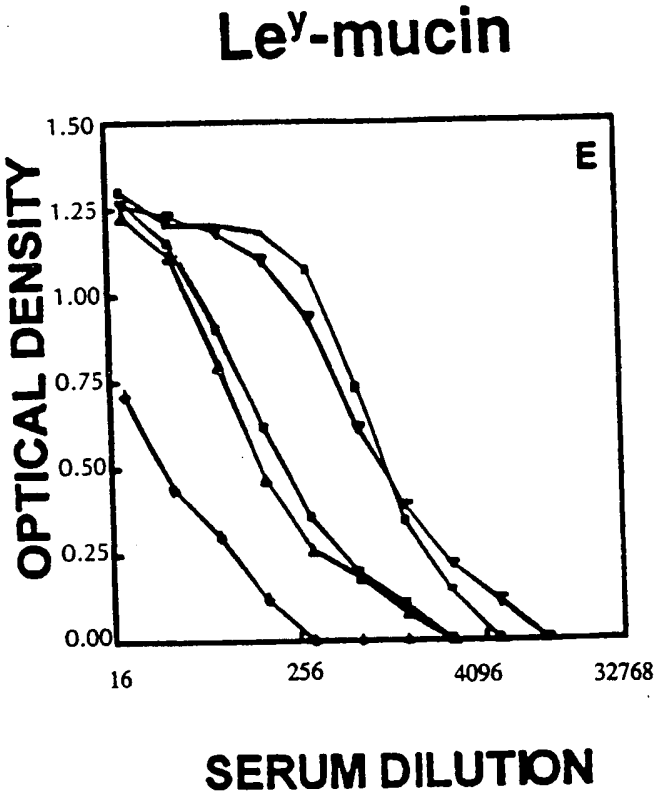
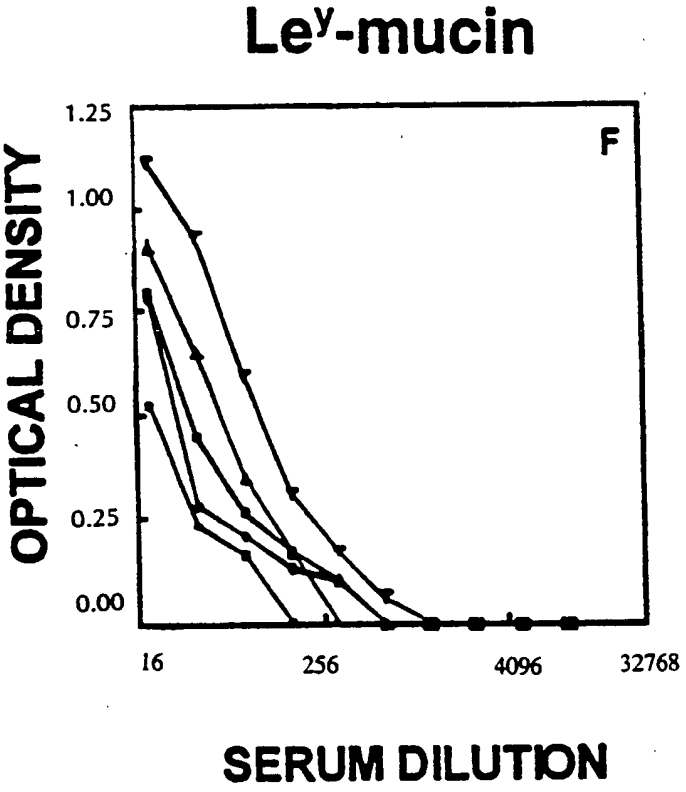

Le^y-mucin
E
OPTICAL DENSITY
1.50
1.25
1.00
0.75
0.50
0.25
0.00
16
256
4096
32768
SERUM DILUTION

FIG. 31F


Le^y-mucin
F
OPTICAL DENSITY
1.25
1.00
0.75
0.50
0.25
0.00
16
256
4096
32768
SERUM DILUTION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/06976

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00
US CL :514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 185.1, 193.1, 194.1, 277.1; 514/2, 8, 23, 25, 506, 510

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, 5,212,298 A (RADEMACHER et al.) 18 May 1993, see especially the N-GLYCOSIDIC LINKAGE TO PROTEIN diagram in column 1.	1-58 and 58 (2nd)
A	US 5,280,113 A (RADEMACHER et al.) 18 January 1994, see especially the chemical structure given in column 1.	1-58 and 58 (2nd)
A	US 5,421,733 A (NUDELMAN et al.) 06 June 1995, see especially the abstract and Figures 1A through 6 and Figures 11 through 14.	1-58 and 58 (2nd)

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
16 JUNE 1999	09 JUL 1999
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International application No.
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B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS, EMBASE, MEDLINE, BIOTECH ABS, WPI search terms: alpha, link,glycoconjugate, peptide, trimeric, antigenic, vaccine